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The associations between sugar sweetened beverage intake, satiety, and metabolic health in minority youth

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Dedication

This work is dedicated to the young Black and Hispanic communities, in loving memory of Sandra Bland and the victims of the Orlando Massacre.

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The associations between sugar sweetened beverage intake, satiety, and metabolic health in minority youth

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Non-Hispanic Black (NHB) and Hispanic children are at an increased risk of obesity and metabolic disease in the United States. Increasingly obesity is viewed as a multifaceted phenotype, beyond simply excess body weight. Research indicates that homeostatic mechanisms as well as hedonic systems are altered in obese individuals. The objective of this study was to examine how sugar sweetened beverage (SSB) intake, a highly modifiable dietary behavior, impacts both metabolic health and hedonic perceptions. This study is made up of three cross sectional analyses: the first two are secondary analyses of data from the University of Southern California, and the third was preformed at the University of Texas at Austin. The first examined the relationship between SSB intake, perceived hunger and satiety, and endocrine biomarkers in overweight and obese NHB and Hispanic adolescents (14-17 y). SSB intake of two servings or more per day was associated with decreased satiety and suppressed ghrelin compared to subjects that consumed one or less servings of SSB. The second examined the association between SSB intake, visceral fat accumulation (VAT), and cortisol awakening response (CAR) in a similar sample of 60 overweight and obese NHB and Hispanic adolescents (14-17y). SSB intake of two servings or more was associated with increased VAT and increased CAR compared to subjects that consumed one or less servings of SSB. The third examined the effect of SSB intake on reward pathways in the brain using functional

magnetic resonance imaging (fMRI), as well as how free-living dietary and dietary intake at an ad libitum meal impacts hunger and metabolic biomarkers in 41 overweight and obese Hispanic children (7-10 y). Although analysis of the fMRI data was uninterruptable, increased hunger and decreased satiety at an ad libitum meal was related to added sugar intake greater than 10% of the subject's daily calories. In adolescents, SSB intake is associated with decreased feelings of fullness and an unfavorable metabolic profile. In young children, added sugar intake is associated with appetite independent of homeostatic factors.

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List of Abbreviations

US- United States
NHB- Non-Hispanic Black
NHW- Non-Hispanic White
BMI- Body mass index
y- Years of age
T2D- Type 2 diabetes
VAT- Visceral adipose tissue
SAT- Subcutaneous adipose tissue
NAFLD- Non-alcoholic fatty liver disease
CVD- Cardiovascular disease
SSB-Sugar sweetened beverages
USC- University of Southern California
UT- University of Texas at Austin
HDL- High density lipoprotein
LDL- Low density lipoprotein
VLDL- Very low density lipoprotein
PYY- Peptide tyrosine tyrosine
HFCS- High fructose corn syrup
SI-Insulin sensitivity
fMRI-Functional magnetic resonance imaging
HOMA-IR- Homeostasis model assessment of insulin resistance
RBP4- Retinol binding protein 4
D2R- Dopamine 2 receptor
BOLD- Blood oxygenation level dependent
CBF- Cerebral blood flow
HPA- Hypothalamic pituitary axis
CTU- Clinical trials unit
PSS- Perceived stress scale
NDS-R- Nutrition data system for research
AHA- American Heart Association
ml- Milliliters
VAS- Visual analog scale
BET-Brain extraction tool
MCFLIRT- Motion correction FMRIB's linear image registration tool
TR- Repetition time
TE- Echo time
MP-RAGE- Magnetization-prepared rapid-acquisition gradient echo sequence
FD- Framewise displacement
FNIRT- FMRIB's Non-linear Image Registration Tool
MNI- Montreal Neurological Institute
PE-Prediction error

TFCE- Threshold free cluster enhancement
SVC- Small volume corrections
ROI- Region of interest

Chapter1: Introduction

The United States (US) Census Bureau projects in 2060 the Non-Hispanic Black (NHB) population will have increased 63% and the Hispanic[1] population will have increased a dramatic 115%[1]. Despite the staggering increase in NHB and Hispanic populations in the US, both still become ill at younger ages and die earlier compared to Non-Hispanic Whites (NHW) [2]. This inequality is apparent in the prevalence of obesity. Risk of becoming obese is disproportionately higher in ethnic minority groups, Hispanic adults have 1.81 increased odds of being obese as adults, and NHB have 1.97 increased odds of being obese as an adult compared to NHW [3]. Despite the overwhelming increase risk of early death and obesity, NHB and Hispanic populations are exceedingly underrepresented in scientific research [4].

Obesity also disproportionately impacts youth, with 53% of NHB children (2-19 years of age (y)) and 39% of Hispanic children overweight (Body Mass Index (BMI) ≥85th percentile for sex and age) or obese (BMI ≥95th percentile for sex and age) in the US, compared to 28% of NHW children [5]. Childhood is a critical period in the development of overweight and obese status [6]. Hispanic overweight and obese children in particular are at an increased risk of type 2 diabetes (T2D), beta cell dysfunction, nonalcoholic fatty liver disease (NAFLD), insulin resistance, cardiovascular disease

[1] The author would like to acknowledge that Hispanic is an ethnicity and that Hispanic people can be any race. For the purpose of this paper Hispanic includes all ethnicities from Central America, South America, and the Caribbean. Non-Hispanic Black includes all people who self identify as Black but do not consider themselves Hispanic (according to the criteria above). Elsewhere in the paper more specific descriptions are given when available.

(CVD), and inflammation [7–13]. Additionally, risk factors associated with obesity starting in childhood develop through adulthood manifesting as CVD and T2D [14]. NHB and Hispanic Americans are 2.3 and 1.5 times respectively more likely to die from T2D [15]. Thus it is critical to prevent and treat childhood obesity in these populations.

Obesity is a multi-faceted phenotype beyond weight status involving homeostatic mechanisms (endocrine functions and fat partitioning), as well as hedonic systems (hunger, satiety, and reward learning) [16]. Understanding how the body balances homeostatic signals with hedonic desires is critical to the study and ultimately the prevention and treatment of obesity. Perceived hunger, or lack of satiation, is a known barrier for weight loss [17], as well as a possible risk factor for developing obesity [18]. For half a century the Mayer's glucostatic theory has been the preeminent hypothesis for food intake [19]. The glucostatic theory posits that as blood glucose decreases, hunger increases. Conversely, when blood glucose is restored to normal values, hunger decreases, and this process is regulated through the hypothalamus [19]. However, in the general population, hypoglycemia is signaled by shaking, sweating, and weakness often without the person feeling "hungry" [20]. Conversely perceived hunger is more closely associated with gastric distension, circadian rhythms (feeling hungry because it is the normal meal time), and food reward learning (feeling hungry because something looks desirable) [20]. Therefore, perceived hunger appears to be different from the homeostatic need to control blood glucose, and seems to be closer to a hedonic system rather than a homeostatic one.

Cognitive systems have been implicated in nutrient intake, weight status, and perceived hunger [21]. Desire to eat pleasurable foods is an impediment to weight loss in

children [22,23]. Hedonic reward has previously been speculated to override homeostatic satiety inputs, such as those outlined in the glucostatic theory, leading to overconsumption of palatable foods and drinks leading to weight gain and maintaining a positive feedback system preventing weight loss [16]. On the surface, hedonic reward is commonly thought of as analogous to pleasure, however reward is a multidimensional psychological component comprised of learning, incentive motivation, and pleasure [24]. Learning about rewards is critical for survival. Being able to regularly predict a good outcome from a poor outcome is important for everything from regularly finding calorie dense food to choosing a good investment. Prediction error encodes learning about rewards, updating the brain with new information when necessary, and filtering out expected responses. Within the brain, prediction error can be quantitatively measured via dopamine signaling [25–28]. When a stimulus is better than expected, it results in higher dopamine response, when a stimulus is poorer than expected it attenuates the dopamine response [29]. Therefore, the effect of the stimulus when it is different than expected is a useful measure of reward related learning [30]. With consistent learning (pairing of a cue with a reward), the reward response becomes habituated, and the reward no longer elicits a response in the brain; rather the reward response in the brain is paired to the cue [31]. In animal models, this habituated response can induce feeding in satiated animals [31]. This raises the possibility that an obesegenic environment, one filled with food cues such as advertisements and easily accessible food, habituates people to the rewarding aspects of food, and therefore promotes eating in the absence of hunger. Overweight and obese

people, and children in particular, may be more susceptible to this habituation. More research is needed to examine food reward learning in children.

The concept of habituation raises the idea that if the brain can be habituated to a stimulus then possibly other physiological systems can also undergo habituation. Obesity may be a physical manifestation of whole body habituation to satiety related endocrine stimuli. Ghrelin, secreted from the stomach, produces a strong orexigenic response. Interestingly, as body weight increases ghrelin secretion is decreased, and this relationship has been shown in adults [32], adolescents [33], and children [34]. Although ghrelin signaling is during weight gain decreased the desire to eat remains, suggesting that the body may become habituated to ghrelin signaling in obesity. Inversely to ghrelin, leptin is an anorexic hormone secreted from fat cells, and it increases with fat mass [35]. Over time in obese subjects, leptin sensitivity breaks down resulting in leptin resistance [36]. The combination of low ghrelin and high leptin in obesity suggests habituation to endocrine satiety signaling in obesity.

Additionally, leptin, and other adipocyte-specific hormones, represent a unique endocrine system. Adipose as an "organ" has the ability to considerably increase or decrease mass through out the lifespan. Moreover, the fat "organ" is not a collection of cells in one location working in concert (like the liver or pancreas), rather the partitioning of fat is related to the endocrine activity of the adipocytes [37–39]. Visceral adipose tissue (VAT) is the fat found around the abdominal organs in the mesentery and omentum, where as subcutaneous adipose tissue (SAT) is found under the skin [39]. Not only are they different in the physical location in the body, but also VAT and SAT are also functionally

and metabolically different. High VAT is associated with an increase in leptin and other pro-inflammatory proteins, and a decrease in the anti-inflammatory adiponectin [39,40]. VAT is important not only for the hormones it produces, but also for the hormones it interacts with, such as the adrenal-produced hormone, cortisol. Interest in the relationship between cortisol and VAT comes from the pathological elevation of cortisol seen in Cushing's disease [41]. People suffering from Cushing's disease exhibit high VAT depots along with insulin resistance and depressed high density lipoprotein (HDL) levels [42,43], which suggests that the high cortisol levels might induce the metabolic disease risks. VAT contains a higher proportion of cortisol receptors, which transform the inactive cortisone to the functional hormone cortisol [44]. In animal models, this local activation has been linked to further increases in VAT [44]. In children and adolescents, systemic cortisol has been associated with higher fat mass and insulin resistance [45], and higher morning serum cortisol has been linked with increased prevalence of metabolic syndrome [46].

The alterations in endocrine concentrations seen in obesity may also influence hedonic reward mechanism in the brain [47]. Ghrelin receptors have been isolated in dopamine producing regions, which has been implicated in overeating and enhancing the palatability of food [48]. In an animal model, hyperleptinemia in the ventral tegmental area resulted in leptin resistance [48]. In a human functional magnetic resonance imaging (fMRI) study, mesolimbic reward areas were associated with plasma leptin concentrations and BMI, which suggested that leptin signaling in the brain may be impaired in obese subjects [49]. Cortisol also has recently been examined as a potential moderator of hedonic eating [50–52]. To date no study has examined the effects of endocrine factors on reward

learning in children. Understanding how VAT, endocrine factors, appetite and reward learning interact together could inform future obesity treatment and intervention programs.

An appealing, and highly modifiable, obesity intervention target is sugar sweetened beverage (SSB) intake [53–56]. SSBs are the main source of added sugar in US children's diets [57]. Minority youth, on average, consume more calories from SSB compared to NHW peers [58]. Consumption of SSB has been shown to increase VAT in adults [59–61], has been associated with reduced beta cell function in Hispanic youth [62], and has been shown to alter reward learning in an animal model [63]. In a randomized 16-week nutrition intervention, focused on reducing added sugar and SSB intake, Hispanic and African American adolescents (14-18 y) showed significant improvements in insulin sensitivity (SI) and inflammation markers [64]. In a 12-week randomized controlled trial decreases in SSB consumption decreased intrahepatic fat in overweight and obese subjects [65]. Preliminary findings show that SSB-based interventions are feasible and generally well tolerated in adults and children [66–69]. Examining the effect of SSB on endocrine systems, fat partitioning, and reward learning is important in creating meaningful interventions in overweight/obese pediatric populations, particularly in high-risk minority youth populations.

A key consideration in understanding the effect of SSB on the body and brain is the understanding the physiological role of the primary sweetener, high fructose corn syrup (HFCS). The use of HFCS as a sweetener has increased over 1000% in the last three decades, and makes up approximately 40% of caloric sweeteners [70]. Unlike sucrose,

which is molecularly 50% fructose and 50% glucose, HFCS is a combination of free fructose and free glucose in varying proportions determined by the individual manufacturer [71]. The proportion of free fructose in HFCS was found to be as high as 65% in popular SSBs in the US, which means there is nearly 50% more fructose in HFCS compared to sucrose [72,73]. Stanhope and colleagues have preformed a series of elegant experiments in humans describing the effect of fructose consumption on metabolic health, showing that high fructose consumption increased CVD risk factors, liver fat, *de novo* lipogenesis, and VAT [74–76]. A recent fMRI study showed that fructose consumption compared to sucrose consumption resulted in different cerebral blood flow in the hypothalamus [77], however it remains unclear what that difference means in terms of food intake, satiety, or reward learning. Metabolically, fructose from HFCS was shown to be more bioavailable than fructose from sucrose [78]. However, studies comparing sucrose to HFCS found no difference between HFCS and sucrose on endocrine factors in adults [76,79–86]. Obese adolescent subjects had depressed ghrelin response to fructose compared to lean adolescent subjects [86]. While many of the above studies have examined the acute effects of either fructose or HFCS, to date no work has examined the long-term exposure to HFCS. Additionally, no study has looked holistically at the effect of both a HFCS stimulus and free-living HFCS intake on satiety, metabolic parameters, or reward learning in humans.

This project was comprised of a two secondary analyses of cross-sectional data from the University of Southern California (USC) and a cross-sectional experiment at the University of Texas at Austin (UT). The first secondary analysis examined an acute

crossover feeding trial, comprised of 47 overweight, African American and Hispanic youth (14-17 y). The subjects were given a high sugar breakfast and lunch on one day and a low-sugar breakfast and lunch on a separate day, both followed by an *ad libitum* dinner. Energy intake at the *ad libitum* meal and metabolic hormones (ghrelin, peptide tyrosine tyrosine (PYY)) were measured. The second secondary analysis examined the relationship of SSB consumption on fat partitioning and cortisol response in 60 African American and Hispanic youth (14-17 y). The third experiment was a cross-sectional study, which examined the relationship between SSB intake, metabolic health, and reward sensitivity in a sample of 41 overweight and obese Hispanic children (7-10 y).

The overarching goal of this project is to evaluate the effects of sugar sweetened beverage consumption on endocrine biomarkers, satiety, fat partitioning, and reward learning in overweight and obese minority youth (7-17 y). The overarching hypothesis is that increased SSB intake in minority youth is related to decreased satiety, increased VAT, aberrant endocrine patterns, and blunted reward learning.

Chapter 2: Literature Review

CHILDHOOD OBESITY

"Baby fat", "big for his or her age", "waiting for a growth spurt," are placating phrases mollifying an epidemic: childhood obesity. From 2011- 2014, 17% of children (2-19 y) were obese (BMI ≥95th percentile), with the highest rates (20.5%) in adolescents (12-19 y)[5]. Children (6-9 y) who are obese have a 10 times greater risk of becoming obese as an adult compared to non-obese children, and this risk skyrockets in adolescences with obese adolescents (15-17 y) having 20 times greater risk of being obese adults compared to their non-obese peers [87]. Obesity in childhood may continue through adulthood and increases the risk of chronic disease. From 1960 to 2005, the percentage of children with chronic disease in the US quadrupled, with minority youth having a higher likelihood for diseases [88]. Childhood obesity has been associated with a clustering of CVD risk factors[89,90], and is itself a risk factor for developing T2D, hypertension, dyslipidemia, and atherosclerosis [5,91], and overall metabolic syndrome [92].

IMPACT OF CHILDHOOD OBESITY IN NHB AND HISPANIC COMMUNITIES

The need for research into prevention and treatment of childhood obesity in minority populations is paramount. NHB and Hispanic minorities are disproportionately at risk for obesity, with 35% of NHB and 38% of Hispanic youth (12-19 y) being overweight or obese (BMI ≥85th percentile) compared to 28% of NHW youth [5]. NHB and Hispanic children also have higher central fat and overall fat volume compared to NHW

peers [93]. Additionally, NHB and Hispanics are at increased risk of chronic disease in childhood [88], in particular T2D and metabolic syndrome [94,95]. Obese NHB children are at higher risk of having insulin resistance, CVD, and T2D compared to obese NHW children [13]. Hispanic overweight and obese children in particular are at an increased risk of T2D, beta cell dysfunction, nonalcoholic fatty liver disease (NAFLD), insulin resistance, inflammation [7–12], and metabolic syndrome [96] compared to NHW children. Considering that as adults NHB and Hispanics are 2 and 1.5 times as likely to die from diabetes compared to NHW respectively [2], treatment and prevention of obesity in childhood in these minority population is crucial.

Obesity in childhood is not a homogenous process. Different stages of childhood have different risk factors and different obesity prevention/intervention targets. In Texas, weight gain increased with age in Hispanic children peaking at 10 y in girls and 11 y in boys [97], indicating that intervention for weight management is needed during young childhood. Conversely, adolescence is a time of increasing autonomy when youth begin to make independent dietary choices[98], and therefore is also an ideal time to address obesegenic behaviors.

SSB INTAKE AND WEIGHT GAIN THROUGHOUT CHILDHOOD

Throughout childhood and into adolescence a common target for obesity intervention is SSB intake. SSB intake is a highly modifiable obesity risk factor [53–56], and is the main source of added sugar in US children's (2- 18 y) diets [57]. While overall consumption of SSBs have decreased modestly (3%) in the last decade, daily energy

intake from SSB still accounts for 10% of daily calories [99], with NHB and Hispanic youth consuming on average more calories from SSB than their NHW youth peers [58]. High school students are more likely to consume SSB compared to middle and elementary school children [100], suggesting that SSB interventions must focus on reducing intake in childhood and preventing intake as teenagers.

A recent meta-analysis examining the effect of SSB intake on obesity found a positive association between SSB intake and obesity in children [53]. In a seminal paper, Ludwig and colleagues found that with each additional increase in SSB servings per day, there was a 60% increase in odds of becoming obese [54]. Additionally, the relationship between obesity and SSB intake exists in very young children. Children (2-3 y) who were overweight and consumed one to two servings of SSB per day were twice as likely to become obese at a one year follow up [101]. Young children (2-5 y) who drank SSB were also found to have an increased BMI z-score compared to children who did not drink SSB [102]. A longitudinal study of 365 NHB children (3-5 y) showed that SSB intake was associated with an increased incidence of obesity over 2 years [103]. A Canadian study followed children from birth to 4.5 y, and found that daily consumption of SSB doubled the odds of becoming overweight at 4.5 y [104]. Research suggests that simple changes can prevent obesity. A randomized control trial of obesity prone children (2-6 y) found that replacing SSB intake with milk was inversely related to weight gain [105], therefore a modest substitution from SSB to a lower sugar drink such as milk is an effective method to reduce weight gain. In young children (2-5 y), SSB intake appears to accelerate the increase in the incidence of obesity, with obesity incidence increasing in as little as one

year.

In slightly older children (8-11 y), SSB intake has been associated with an increased BMI and body fat mass in children (8-11 y)[106,107]. A 0.22 mm increase in waist circumference over a period of a year per additional teaspoon of liquid added sugar[108]. Since an 8-ounce serving of SSB contains 48 teaspoons of liquid added sugar that represents just over a 10 mm increase in waist circumference. While consumption of traditional SSBs (carbonated sodas) has decreased, non-traditional SSB (sports drinks and energy drinks) have increased [99] and pose just as serious a risk to health as their traditional counterparts. In children (9-16 y), each serving of sports drinks (Gatorade, Powerade, etc) was associated with an increase of 0.3 BMI units over 2 to 3 years [109]. In addition to the high risk of obesity, in female children (9-14 y) 1.5 servings of SSB per day was related to earlier onset of menarche, and this effect was seen across levels of BMI [110]. Earlier onset of puberty has been associated with a host of psychological and cancer related disadvantages [111–113] as well as increased risk of adult obesity and T2D [114]. Adolescence is a time marked by the transition from pre-puberty to puberty as well as a time of increasing personal autonomy, especially over dietary choices [115]. High school students are more likely to consume SSB compared to middle and elementary school students, and NHB and Hispanic high school students consume higher amounts than NHW [100]. Adolescence may also be a time of sensitivity to weight gain from SSB. A longitudinal study showed SSB was associated with increased BMI and waist circumference in teenagers (15 y) over a period of six years [116]. Additionally, SSB intake has been associated with increased body weight in adolescents in multiple studies [117–119],

with a dose responsive relationship between SSB intake and obesity risk in adolescents who consume four or more eight ounce servings per week [120]. Overwhelming evidence indicates that SSB intake across all ages in childhood is linked to increased adiposity rates although how is it increases adiposity and metabolic syndrome is not understood.

SSB intake has also been shown to cluster with multiple other risk factors for obesity during adolescence such as: increased sedentary activity[121], decreased physical activity [122], increased intake of fried foods and desserts, decreased intake of fruits and vegetables [123], and an increase in overall snacking [124]. In a large sample of children (6-12 y), SSB intake and being less physically active were associated with increased risk of obesity [125]. Specifically in Hispanic children (10-14 y), physical activity and SSB intake were noted as key variables in insulin resistance risk as well as obesity risk [126]. Similar results were seen in adolescents (12-19 y), with SSB intake and physical activity associated with insulin resistance [127].

Adolescences may be an ideal period for SSB intervention. Ebbeling and colleagues performed a randomized control trial in 224 overweight and obese adolescents assigning them to a one year SSB intake intervention. At the one year follow up, the SSB intervention group showed significant weight reduction compared to the SSB consumers and the intervention effects were significant at a two year follow up in the Hispanic subjects [128]. Another small pilot intervention in teenagers (13-18 y) also found that decreasing SSB intake decreased BMI, and subjects with higher BMIs saw more improvement in the intervention [119]. Therefore, interventions focusing on reducing SSB intake have shown efficacy in reducing obesity levels.

Research shows that SSB intake is not exclusively related to increased obesity levels, but also related to a host of other metabolic disturbances. SSB intake has been associated with increased CVD and stroke risk factors in adults[129], adolescents [130,131], and particularly in Hispanic populations [132]. A recent study in Hispanic children (10-14 y) found SSB consumption was positively associated with both the child's BMI and insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR) [110]. A study of European children (12.5-17.5 y) showed similar results, with SSB intake positively linked to both BMI and HOMA-IR [133]. The metabolic syndrome (which is defined as insulin resistance, visceral adiposity, atherogenic dyslipidemia, and endothelial dysfunction [134]) has also been linked to SSB intake [55]. A study of 1454 adolescents (12-16 y) found that SSB intake was positively associated with serum insulin, HOMA-IR, waist circumference, and serum uric acid; additionally these results were more pronounced in overweight and obese subjects [135]. One study found an association between SSB intake and metabolic syndrome independent of total sugar intake and adiposity [56], indicating that SSB may be a unique dietary factor related directly to metabolic syndrome. Determining the nature of the association between SSB intake and metabolic diseases is important for obesity interventions.

EFFECT OF FRUCTOSE

A significant element in understanding the effect of SSB on the body is primary sweetener: high fructose corn syrup (HFCS). The use of HFCS is ubiquitous. From 1970 to 1990, the use of HFCS has increased 1000% and represents over 40% of the caloric

sweeteners added to food [70]. US children (2 y and older) get an average of 132 calories per day from HFCS, and the top consumers get on average 316 calories per day [70]. Confusion has plagued the US public about what HFCS is and is not, especially in comparison to sucrose. Both sucrose and HFCS are made of the same basic components, fructose and glucose. The difference arises in the proportion of fructose to glucose. Sucrose is molecularly bound 50% fructose and 50% glucose and is solid at room temperature [71]. Whereas HFCS is a combination of free fructose and glucose and is a liquid at room temperature [71]. The proportion of free fructose to glucose in HFCS is dependent on the manufacturer [71]. A study out of the University of Southern California (USC) found that the proportion of fructose in popular US SSB was as high as 65%, which means there is nearly 50% more fructose in SSB sweetened with HFCS compared to sucrose [72,73]. This is particularly worrisome given the literature on the effects of HFCS on metabolic health.

Stanhope and colleagues have documented the effects of isocaloric fructose consumption compared to glucose consumption in obese adults. Over a 10 week period, fructose increased VAT, triglyceride concentrations, hepatic *de novo* lipogenesis, low density lipoproteins (LDL), plasma glucose, and plasma insulin [75]. Similar results have been reported in adolescents, the largest consumers of fructose [136], total dietary fructose was positively related to increased visceral adiposity, increased blood pressure, and HOMA-IR [137]. Furthermore, overweight and obese adolescents who consumed 500 ml per day of beverages sweetened with HFCS had higher triglycerides and retinol binding protein 4 (RBP4), a protein shown to be related to insulin resistance [138], as well as higher

blood pressure and serum uric acid [78]. While the effect of fructose on metabolism is well documented, the effect of fructose and HFCS on energy intake and satiety is mixed with studies finding an increase in caloric intake after a HFCS preload [139] or having no effect on *ad libitum* intake [84]. While all added sugars should be reduced, HFCS (due to a high proportion of fructose) may be more metabolically harmful and therefore should be limited.

HUNGER: HEDONIC OR HOMEOSTATIC?

Hunger is a noted barrier to weight loss in adults [17] and children [22,23], as well as a possible risk factor for developing obesity [18]. However, what hunger is and how it is recognized is poorly understood. Generally, hunger is recognized as a motivating factor for consuming food. For half a century the Mayer's glucostatic theory has been the preeminent hypothesis for food intake [19]. The glucostatic theory posits that as blood glucose decreases, hunger increases. When blood glucose is restored to normal values, hunger decreases, and this process is regulated through the hypothalamus [19]. However, in the general population hypoglycemia is signaled by shaking, sweating, and weakness often without the person feeling "hungry" [20]. Conversely perceived hunger is more closely associated with gastric distension, circadian rhythms (feeling hungry because it is the normal meal time), emotional and stress eating, and food reward learning (feeling hungry because something looks desirable) [20]. Thus, hunger, for a majority of the US population, is not purely homeostatically driven, but rather is a product of environmental signals and desires and is better described as perceived hunger. Carnell and Wardle

suggest an obese phenotype in children comprised of low responsivity to internal satiety signaling (homeostatic hunger) and increased responsivity to external food cues (perceived hunger) [140].

IS A CALORIE A CALORIE?

In 2004, Buchholz and Schoeller stressed that indeed, "a calorie is a calorie," at least in terms of thermodynamics [141], and together with Mayer's glucostatic theory [19], raises the question of why does SSB appear to independently influence metabolic status as outlined above? Again this comes back to the interaction between homeostasis and hedonic feeding. In a sophisticated cross-over feeding trail, DiMeglio and Mattes showed that liquid carbohydrates compared to isocaloric solid carbohydrates promoted positive energy balance in adults [142]. Further research into liquid calories, such as SSB, indicated that SSB is generally not compensated for at later meals [143], and that intake of total calories is greater for SSB consumers than can be accounted for by just the addition of the SSB [144]. Additionally, both adults and adolescents have been shown to have poor ability to estimate caloric intake when buying beverages compared to when buying solid snacks, consistently underestimating the caloric content of the beverages [145]. These findings suggest that SSB not only adds calories directly, but may also increase caloric intake from other sources. In adults, SSB intake was correlated with choosing foods high in sugar [146], further suggesting SSB consumption may be influencing satiety factors to increase overall calorie intake.

Low satiety (the psychological sensation of lack of fullness [147]) has been related to

overeating, which is a factor in developing obesity [148]. Jansen and colleagues trained (repeatedly exposed) normal weight and obese children (8- 12 y) with a palatable food preload, and measured appetite and energy intake at a preceding meal. They found the obese children, compared to the normal weight, did not compensate for energy intake at the meal after a palatable preload [149]. Similar results were seen in younger children (3-5 y), children with high adiposity did not compensate for energy intake at a test meal after a caloric preload [140]. These studies suggest that in children, caloric intake is uncoupled from energy need and may be driven by palatability or by a feeling of obligation to eat the food given [150–153]. In a large sample of children (n=10,364; 8-11 y and 3-5 y), higher BMIs were associated with lower satiety responsiveness and this association was stronger in the older children [154]. This study suggests that satiety is related to body composition and that this relationship increases with age, further indicating that research into young children's appetite behaviors is critical. In male children (9-14 y), acute administration of a sucrose beverage preload increased appetite at an ad libitum lunch; however, a glucose beverage preload reduced food intake indicating a difference between the two sugar types [139]. Cassady and colleagues found that oral liquid preloads result in greater post-prandial hunger and lower fullness sensations and increased gastric emptying [155]. The increased gastric emptying could be a mechanism by which liquids fail to induce satiety. Gastric distension is critical for the psychological feeling of satiety [147]. A lack of gastric distension from liquids containing calories could produce higher caloric intake and decreased satiety. In support of this hypothesis, consumption of a caloric beverage with a meal did not influence short term satiety but did increase the overall calorie intake

at the meal [143]. At the very least SSB intake provides additional calories and added sugars, at worst SSB hijacks satiety systems and increases perceived hunger.

HUNGER AND REWARD LEARNING

Perceived hunger is closely associated with desire to consume palatable food [156]. Yeomans and colleagues fed human subjects either a high or low energy soup preload and the preload was followed by either a palatable or unpalatable lunch. Subjects given a high calorie preload reported less hunger before the lunch compared to the low calorie preload. However when presented the palatable food, both high and low calorie preload groups reported higher hunger [156] showing that hunger was initially related to energy content, but in the face of palatable food, the desire to eat increased perceived hunger.

To understand perceived hunger, obesity researchers are looking to addiction research, psychology, and cognitive neuroscience methods for insight [21]. Intake of palatable food is a hedonic reward: a multidimensional psychological component comprised of learning, incentive motivation, and pleasure [24]. Learning about rewards is critical for survival. Being able to regularly predict a good outcome from a poor outcome is important for everything from regularly finding calorie dense foods to choosing a good investment. Prediction error encodes learning about rewards, updating the brain with new information when necessary, and filtering out expected response. Within the brain this can be quantitatively measured via dopamine signaling [25–28]. When a stimulus is better than expected it results in higher dopamine response, when a stimulus is poorer than expected it attenuates the dopamine response [29]. Therefore, the effect of the stimulus

when it is different than expected is a useful measure of mechanisms related to reward related learning [30].

Prediction error reward is vital for instrumental learning (operant conditioning) in which a behavior is updated through the result of an action [157]. Instrumental learning is comprised of two mechanisms: habit-based learning (model-free) and goal-oriented learning (model based) [158]. Goal oriented learning evaluates the actions needed to achieve an anticipated outcome (goal) according to the state (both internal homeostasis and external environment), whereas habit based learning repeats an action based on a previous response regardless of current conditions [158]. Habit based learning is faster, less computationally intensive, and more automatic [158]. However, it can lead to making decisions not in the subject's best interest, such as continuing to partake in a self-harming behavior due to previous rewards. Habit based learning has previously been implicated in addiction, where an individual does not or cannot evaluate his or her current condition, but rather uses the previous response experience to make a decision and is insensitive to positive or negative reinforcement resulting from the decision [159]. Insensitivity to positive or negative reinforcement was seen in a pivotal paper by Johnson and Kenny, where obese rats exhibited compulsive-like over consumption of palatable food, which was resistant to aversive conditioning (pairing the palatable food with a negative event such as a shock), and additionally showed down regulated striatal dopamine 2 receptors (D2R) compared to lean controls [160]. Further work has shown that down regulation of D2R and compulsive-like behaviors (overeating in the absence of hunger) can be induced via "overtraining," which makes the behavior unreceptive to extinction (the decrease in a

behavior when it is no longer rewarding) [159]. Therefore, Johnson and Kenny's obese rats could be viewed as "over trained" to consume palatable food, and that this overtraining mimicked a neuro-maladaptive response similar to substance abuse [160]. In another animal study, Sharpe and colleagues showed that habitual exposure to sucrose acted as an overtraining influence [63]. Overtraining from habitual exposure to sucrose impaired the animals' abilities to distinguish rewarding and non-rewarding stimuli compared to those not exposed to sucrose [63]. In human children, exposure to a food cue (a "tasty" smell) increased food consumption in overweight children compared to normal weight [149], demonstrating overweight status as a correlate for the over-trained mice in Johnson and Kenney's experiment. It is possible that an obesegenic environment, one filled with food cues such as advertisements and easily accessible food, habituates people to the rewarding aspects of food, and therefore promotes eating in the absence of hunger. Obesity could be considered a physical manifestation of the shift from goal-directed learning to habit-based learning, characterized by high perceived hunger and low sensitivity to internal satiety signaling as suggested by Carnell and Wardle.

METABOLIC HABITUATION

Insulin

The obese phenotype defined above is made up of two distinct components, perceived hunger and internal satiety signaling, which are intertwined and therefore share similar mechanisms. As dopamine receptors can undergo habituation, it stands to reason that physical systems can also undergo a form of habituation. The most noticeable correlate of physical habituation is insulin resistance, in which an amount of insulin no

longer elicits a biological response [161]. Insulin resistance is considered the link between obesity and metabolic syndrome [162]. Classically, insulin resistance is characterized by the inability of insulin to signal for glucose uptake and suppress hepatic glucose production, resulting in both hyperinsulinemia and hyperglysemia until the beta cells "burn out" and can no longer keep up with insulin production resulting in hypoinsulinemia [161]. Insulin resistance has been shown to be more prevalent in minority populations [94], particularly in Hispanic and NHB children [7,9,64,163–165]. While the effect of insulin resistance on glucose uptake is well known, the effect of insulin resistance on perceived hunger is less understood. Insulin is known to impart an anorectic response under normal conditions [166]. The habituation hypothesis suggests that, in an insulin resistant state, insulin would fail to impart its anorectic signal and perceived hunger would persist. Preliminary research seems to support this hypothesis. Studies between lean and obese humans indicate that in lean individuals insulin produces a satiating response, however this response is not seen in the obese [167–169]. A study of obese and lean humans found that HOMA-IR levels were positively correlated with neural activity in dopaminergic brain areas and with food craving [170]. Additionally, weight loss appears to recover not only insulin sensitivity, but also insulin's ability to promote satiety [171]. Improving insulin sensitivity may provide a two-fold impact on obesity, both improving metabolic health and helping augment satiety to promote weight loss or maintenance although this has yet to be explored in children.

Gut hormones

Many other hormones are known to influence satiety and metabolic health. Ghrelin, the only known orexigenic hormone, is secreted from the stomach and promotes meal initiation and forging behaviors in mammals [172]. In lean human subjects, ghrelin is high when fasting or before meal initiation and drops as food is consumed [172]. Ghrelin has been shown to interact with the dopamine producing areas of the brain [47,48,173]. Ghrelin also increases the blood oxygenation level dependent (BOLD) response in reward and taste areas of the brain [174–179], and has been reported to incentivize the intake of food in animal models [48]. Research in obese subjects indicates that ghrelin also undergoes habituation or resistance with increased fat mass [32,180], and this has been seen across the lifespan [33,34,97,181–183]. Initially, the inverse relationship between ghrelin concentration and body fat appears counterintuitive; one would assume obese individuals have greater hunger and therefore higher ghrelin concentrations. However, the key to aberrant ghrelin concentrations is linked to insulin resistance, as insulin suppresses ghrelin secretion in adults [180,184] and children [166,183,185]. Furthermore, weight loss has been shown to increase ghrelin [186]. The maintenance of hunger in spite of low ghrelin levels further implies that hunger is hedonically and not homeostatically motivated in a habituated state (obesity).

Not only are ghrelin concentrations lower in obese individuals, but also ghrelin response to food is altered in obesity. In Hispanic adolescents (11-14 y), ghrelin suppression was blunted in obese subjects after a meal [187]. So, while overall ghrelin is low, it is also less responsive to food intake, further indicating an uncoupling of the homeostatic need to ingest with food intake. Additionally, ghrelin action seems to be

influenced by macronutrient composition. With high carbohydrate intake in normal weight children (7-11 y), ghrelin reached a nadir faster and rebounded faster [188]. In obese girls (12-18 y), a high carbohydrate breakfast increased ghrelin after the meal [189]. Since SSB are a high carbohydrate breakfast, SSB intake may further alter ghrelin action influencing potential ghrelin habituation. However, the impact of SSB intake on ghrelin responses is understudied.

While a multitude of anorexic hormones exist, peptide tyrosine tyrosine (PYY) has been well studied in children and adolescents and is commonly used as a complementary hormone to ghrelin [190]. PYY is secreted in response to food ingestion from the ileum and colon and has been shown to reduce food intake and impart a feeling of satiety [191]. Like ghrelin, PYY is sensitive to macronutrient intake [190,192–194]. In girls (12-18 y), high fat and high carbohydrate breakfasts did not elicit as high of a PYY response compared to a high protein breakfast [189]. In children (7-11 y), a high carbohydrate breakfast increased PYY faster, but a high protein breakfast resulted in a slower (but longer lasting) increase in PYY[188]. In both children (7-11 y) and female teenagers (12-18 y), obese subjects exhibited a blunted PYY response[188,189,195] and this blunting has been seen in obese Hispanic children (11-14 y) as well [187]. Whether this blunting is a result of obesity or other factors related obesity is unknown. However, the lower PYY response in obese subjects and the lower response to high carbohydrate meals warrant further investigation for possible habituation.

Adipokines

Adipokines, peptide hormones secreted from adipose tissue, are important mediators of body and nutritional status [196]. Continuing with the notion of obesity as a state of long term overtraining, resulting in increased fat mass and habituation to satiety signaling, then adipokines are an output of this "overtraining". The first adipokine discovered, and most researched, is leptin [197], and it has been implicated in satiety signaling , body mass, stress, and glucose metabolism [196]. Leptin is positively related to body fat mass and can be considered a mechanism by which the body relays information about body fat stores to the central nervous system [198]. Like insulin, evidence of habituation is apparent with leptin. Obese individuals with high circulating leptin exhibit "leptin resistance", or a failure of leptin to suppress feeding, specifically at the level of the arcurate nucleus in the hypothalamus [199]. However, the hypothalamus is not the sole area of the brain that leptin influences. Leptin has been shown to predict D2R availability in obese women [200] and diminish dopamine signaling in the ventral tegmental area [201]. In animal models, leptin was also shown to decrease the value of a sucrose reward [202]. Leptin signaling in areas associated with reward further suggests a habituation to leptin outside of the hypothalamus, suggesting palatable food intake is uncoupled from leptin signaling and that intake is based on previous learned associations (habit-based learning) rather than current value (goal-based learning).

Developing research indicates that leptin is an acutely important biomarker in Hispanic populations. Leptin has been shown to be an independent predictor of weight gain in Hispanic children [97,203]. Leptin, and its converse adipokine adiponectin, are related

to insulin sensitivity in Hispanic youth [40,204]. Unlike leptin, adiponectin is an anti-inflammatory adipokine with insulin sensitizing properties in the muscle, liver, and endothelial cells [205]. Despite being secreted from fat mass, adiponectin is inversely related to fat mass [206]. In Hispanic adolescents, adiponectin was inversely correlated with cardiometabolic risk factors [165] and was found to be an independent predictor of metabolic syndrome in overweight Hispanic youth [207]. Leptin and adiponectin appear to be an important markers of metabolic health in Hispanic children.

Increasingly, sugar consumption has been related to disrupted adipokine secretion and signaling. In adults, SSB consumption was positively associated HOMA-IR and increased circulating leptin [208]. Consumption of a fructose or glucose sweetened beverage over 10 weeks suppressed adiponectin, with the largest decreases seen in the group consuming fructose, and this was also associated with abdominal fat [209]. A potential mechanism has been found in an animal model, showing fructose consumption increased VAT, insulin resistance, and adiponectin via the ketohexokinase-dependent pathway in liver, which essential for fructose metabolism [210]. Reduction in SSB intake appears to be an opportune way to decrease dietary fructose and potentially increase adiponectin activity, and reduce insulin, leptin resistance and VAT.

VISCERAL ADIPOSITY AND CORTISOL

Adipose tissue represents a unique endocrine system. Adipose as an "organ" has the ability to considerably increase or decrease size through out the lifespan. Unlike traditional endocrine organs such as the liver or pancreas, the fat "organ" is not a

collection of cells in one location; rather the partitioning of adipose is related to the endocrine activity of the adipose cells [37–39]. VAT, the fat found around the abdominal organs in the mesentery and omentum, opposed to SAT, which is is found under the skin [39], exhibits higher vascularization, and is more metabolically active by secreting adipokines [39].

VAT has been recognized as a useful measurement to assess cardiometabolic risk in adults [211,212] and metabolic syndrome risk in adolescents [213–216]. A minority cohort study demonstrated that VAT accumulation is greatest during young adulthood in NHB and Hispanic populations [217], and therefore adolescence is an important time period to reduce VAT and SSB intake to minimize metabolic syndrome risk. High VAT was related to lower leptin and total adiponectin, increased prevalence of metabolic syndrome [215,218], and increased prediabetes in a cohort of NHB and Hispanic obese teens [219]. In children (mean age 8.6 y), VAT was shown to be related to increased circulating triglycerides, insulin [220], and insulin resistance [221] . Specifically in Hispanic children (8-13 y) with a family history of T2D, VAT was an independent predictor of T2D risk [222].

How VAT is formed, through fat partitioning, is not well understood. Initial research in fat partitioning dates back to 1912 with Harvey Cushing's first description of hypercortisolism now known as Cushing's disease [223]. Cushing noted an "extraordinary appearance" of his patients with a "huge abdomen," which would later become a hallmark sign of Cushing's disease [223]. Since 1912, Cushing's disease has been associated with a host of metabolic syndrome phenotypes [44], suggesting that cortisol plays a critical role in fat partitioning. More recently, research has shown in overweight Hispanic

adolescents that cortisol levels were associated with higher regional fat mass and insulin resistance [45], while morning serum cortisol was negatively associated with fasting glucose, beta cell function, and insulin resistance [224]. Additionally, high free urinary cortisol has been associated with insulin resistance, LDL, HDL, and VAT in overweight girls (12-18 y) [45]. Mechanistic studies in animal models have shown that glucocorticoids (such as cortisol) impair normal adipogenesis favoring VAT production [225], induce hyperleptinemia [226]. In addition, in obese rats, the adrenal glands develop resistance to the inhibitory signaling of leptin [227]. These animal models suggest that the insulin resistance associated with cortisol could be due to the impaired adipogenesis and leptin resistance creating a positive feedback loop for negative metabolic health. Preliminary research indicates that cortisol, VAT, and sugar intake may act synergistically. Gyllenhammer and colleagues found that total and added sugar intake increased the relationship between cortisol and VAT in a sample of overweight/obese NHB and Hispanic adolescents [228]. More research is needed to determine the directionality between sugar intake, VAT deposition, and cortisol.

Along with cortisol, sugar intake appears to play a role increasing VAT [60,137,229,230]. Odegaard and colleagues specifically found that SSB intake was related to an increase in VAT independent of total body fat or BMI [60]. Similarly, Ma and colleagues found daily consumption of SSB in adults was related to 10% higher VAT compared to those who did not drink SSB daily [61]. Similarly, intake of 57 grams or more of SSB was related to 35% higher odds of abdominal obesity compared to those who consumed less than 6 grams of SSB [231]. In a 6 month randomized control trial, subjects consumed 1 liter of

SSB, milk (isocaloric to the SSB), diet SSB, or water. Subjects who consumed SSB exhibited 24-35% higher VAT compared to the other groups [59]. In a large longitudinal study of body fatness in European youth (9-15 y), SSB intake at age 9 y was associated with a larger waist circumference (a surrogate measure for VAT [232]) compared to those who did not drink SSB [116]. Furthermore, a study of 1454 adolescents (12-16 y) in Taiwan found HFCS sweetened SSB intake was related to increased HOMA-IR, and this relationship was at least partially mediated through VAT [135], indicating a relationship between SSB, VAT, and insulin.

The relationship between SSB, VAT, and insulin may also be a driving factor behind metabolic habituation. Again, fructose metabolism provides a potential mechanism. As outlined above, fructose has been shown to increase VAT [75,137]. Fructose is taken up from the portal vein into the liver, where unlike glucose, it by-passes the rate-limiting step of phosphofructokinase. Fructose is then rapidly metabolized by ketohexokinase, which then links to glycolysis at the level of adolase, overloading the glycolytic pathway. This can lead to an increase in *de novo* lipogenesis, and a decrease in hepatic insulin sensitivity [75,210]. Additionally, fructose consumption up regulates very low lipoprotein (VLDL) production in the liver to transport triglycerides synthesized from *de novo* lipogenesis. These VLDL are preferentially stored as triglycerides in VAT [75,210]. Marek and colleagues also found that fructose consumption increased macrophage infiltration of VAT, which may be responsible for the decrease in adiponectin [210] and overall increase in the inflammatory properties of VAT; cortisol may exacerbate this mechanism. As summarized previously, cortisol alters adipogenesis [225], induces

hyperleptinemia [226], as well as increases triglyceride uptake in adipocytes by up-regulating lipoprotein lipase [43]. Therefore the high fructose intake decreases hepatic insulin sensitivity, increases VLDL, and increases VAT inflammation (through increased macrophage infiltration and decreased adiponectin). This state of inflammation may signal for increased cortisol production, which in the obese state leads to increased triglyceride uptake from VLDLs, preferential creation of VAT, and increased circulating leptin, further antagonizing adiponectin and insulin.

FUNCTIONAL MAGNETIC RESONANCE IMAGING AND HOLISTICALLY STUDYING METABOLIC HEALTH

The future of nutritional science is integrative experimentation, by studying how multiple systems work in concert for human health. FMRI offers a non-invasive method of assessing the effect of perceived hunger, SSB consumption, and endocrine balance on the brain. FMRI uses BOLD signaling as a correlate for neural activity, such as dopamine response to prediction error [30]. Much of the current literature has examined the effect of palatable food intake with a milkshake fMRI task (**Figure 2.1** [233]), which for the purpose of this section will be referred to as "the milkshake paradigm".

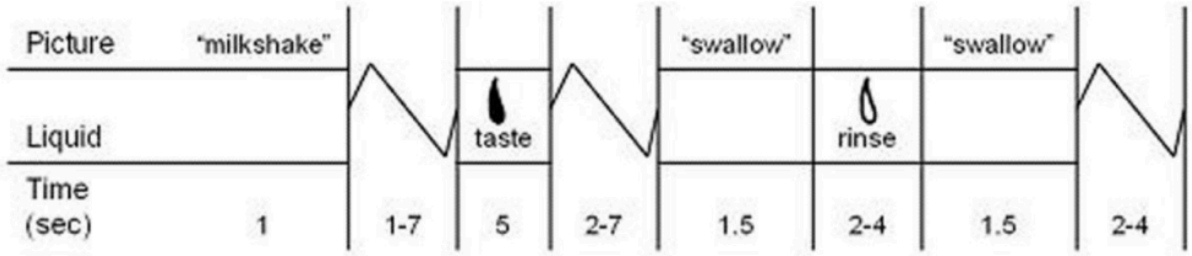

Figure 2.1: The milkshake paradigm

Table 2.1 outlines the current literature using fMRI to assess the effect of various tastes and food imagery on the brain. Research in humans has found that the midbrain appears to mediate the hedonic response to sugar [57,58]. Interestingly, when presented with a sweet reward, obese individuals show a blunted striatal response [57,59], possibly indicating a greater amount of sweet to achieve a reward response. Research in healthy weight adolescent girls who were frequent or naïve ice cream consumers revealed that frequent consumers exhibited a blunted BOLD response in reward related brain regions [16]. Page et al. examined the difference between glucose and fructose stimuli using perfusion analysis in fMRI on the hypothalamus, and appetite and reward regions in healthy, normal weight adults, and found differential cerebral blood flow (CBF) between fructose and glucose consumption [22]. Jastreboff et al. performed a similar experiment in adolescents, showing increased CBF in the striatum after a fructose preload. Obese compared to lean subjects showed reduced connectivity between homeostatic, hedonic, and executive brain regions [234]. Additionally, research has shown that adults will consume a high sugar and fat drink

in the absence of hunger and that the behavior is related to the dopaminergic midbrain [25]. This further implicates a neural compulsion to ingest sweet flavors.

FMRI is also being used in conjunction with endocrine assays. A pilot study in adolescent Hispanic girls found that insulin sensitivity (as measured by a frequently sampled intravenous glucose tolerance test) was inversely associated with BOLD response in brain areas associated with executive function and decision making [235]. In adults, ghrelin and triglyceride circulation were found to be associated with attenuated BOLD response to a milkshake taste in the midbrain and prefrontal cortex [236], and HOMA-IR was positively correlated with cortico-limbic-striatal brain regions while subjects were viewing food cues and stress related cues [170]. Stress may also play an important role in sugar consumption. Tryon and colleagues found that sucrose consumption (compared to aspartame, which is a non-caloric sweetener) was related to increased BOLD response in the hippocampus during an fMRI stress paradigm, and that this association was also related to an increased cortisol response [237]. Considering the mechanism for VAT accumulation and endocrine habituation outlined earlier, increased stress induced cortisol may also promote sugar consumption through hedonic pathways. While multiple studies have examined the effect of palatable drinks on dopaminergic pathways [16-18,58,59,64-71], to date, no research has looked at this relationship using minority children. In addition, the bulk of fMRI and taste research has been conducted in Caucasian, normal weight adults.

Table 2.1 Summary of fMRI experiments looking at food imagery or using taste paradigms

Reference	Population	Paradigm	Main Results
Burger KS, et al (2012) [238]	151 healthy weight adolescents	The adolescents underwent an fMRI during the receipt of a milkshake and a tasteless solution. Body fat, reported food intake, food craving and liking were assessed	Frequent ice cream consumption was related to a decrease response in reward brain regions.
Stice E, et al. (2008) [239]	43 adult women BMI range 23.8–33.2	Milkshake paradigm	Negative correlation between BMI and response in left caudate nucleus to receiving milkshake versus a tasteless solution in both groups. Negative correlation with the putamen and BMI. The Taq A1 allele modified the negative relationship between BMI and left caudate. Obese individuals showed a blunted striatal response and this effect was amplified in A1 alleles.
Burger KS, et al. (2013) [240]	155 adolescents mean BMI 20.8 ± 1.9	Milkshake task was used during scanning. Doubly labeled water was used for the assessment of energy intake and resting metabolic rate. Body composition was assed using a BodPod.	Energy intake was positively correlated positively with BOLD response in: the superior lateral visual cortex, anterior cingulate cortex, frontal operculum, posterior cingulate cortex, precuneus, cunes, posterior middle temporal gyrus, supramarginal gyrus. Adipose tissue was positively associated with BOLD response in attention, gustatory, and reward brain regions when anticipating palatable food.
Gearhardt AN, et al. (2011)[241]	48 adult women BMI range 23.8 - 39.2	Food addiction scores were assessed using the Yale Food Addiction Scale. The milkshake paradigm was used during scanning.	Food addiction scores were related to increased BOLD response in anterior cingulate cortex, medial orbitofrontal cortex, and amygdala in response to anticipated receipt of food. Participants with higher food addiction scores showed increased BOLD response in dorsolateral prefrontal cortex and the caudate in response to anticipated receipt compared those with low food addiction scores.
O'Doherty JP (2002) [242]	8 adults BMI not reported	Subjects presented visual cues were paired with a sweet taste, a salt taste, or a neutral taste.	The anticipation of the sweet taste was associated with BOLD response in the midbrain, posterior dorsal amygdala, striatum, and OFC. The

Continued Table 2.1			OFC was also associated with reward receipt.
Smeets PA (2011) [243]	10 normal weight men	Subjects were scanned twice, before and after a orangeade sweetened with sucrose preload. During the scans subjects were given orangeade sweetened with sucrose or artificial sweetener, milk, and tomato juice.	Before the preload the BOLD response in the amygdala was increased during non-caloric taste of orangeade than by caloric orangeade. Caloric orangeade was associated with BOLD response in the striatum before, but not after the preload.
Spetter MS, et al. (2012) [244]	15 healthy normal weight men	Subjects were scanned twice once after a savory and again after a sweet preload. After scan the savory or sweet preload was offered ad libitum. In the scan, subjects tasted the sweet and savory tastes and rated the pleasantness.	Striatal BOLD response decreased after the sweet preload. Anterior cingulate gyrus predicted ad libitum intake of sweet and savory drinks. Sweet preload was related to an increased BOLD response in the amygdala, midbrain, and ACC.
Veldhuizen MG, et al. (2011) [245]	16 normal weight women	During the scan subjects were given a sweet taste or a tasteless solution after an auditory cue saying, "sweet" or "tasteless." 70% of the trials were matched. After taste, subjects pressed a button indicating which solution they had been given.	Reaction times were faster during the matched trials. Unexpected compared to expected results in in deactivation in the fusiform gyrus. Unexpected compared to expected was associated with increased BOLD response in the thalamus, anterior insula, ventral striatum, OFC, anterior cingulate cortex, inferior frontal gyrus, and IPS. Interaction between stimulus and expectation in the anterior insula.
Sun X, et al. (2014)[176]	32 adults BMI range 19.5-37.0	Scanned with the milk shake paradigm while fasted. Subjects were given milkshake in two different palatable flavors to prevent flavor habituation. After scanning subjects were fed a fixed meal, and an ad libitum meal. Blood was taken to assess metabolic parameters.	Circulating ghrelin and TG were related to attenuated response to milkshake in the midbrain, and dorsolateral prefrontal cortex.
Rudenga KJ, et al. (2012)[246]	14 overweight or obese women	Scanned using the milkshake paradigm and an autobiographical script-driven stress imagery task.	BOLD response in the right amygdala was positively related to cortisol levels. Both the left and right amygdala was associated with milkshake response in the stressful condition. Additionally, there was a positive relationship between stress and BMI with increased BOLD response to the milkshake in the

Continued Table 2.1			OFC.
Rudenga KJ, et al. (2013)[247]	30 adults mean BMI 23	During the scan, subjects tasted different sucrose concentrations ranging from neutral to unpleasant.	Greater response to pleasant sucrose in the ventromedial cortex.
Stice E, et al. (2011)[233]	60 normal weight adolescents, 35 were at high risk of obesity, 25 were low risk	Milkshake paradigm and monetary paradigm were used during the scan.	High-risk subjects showed increased BOLD response in the dorsal striatum in response to milkshake.
De Araujo IE, et al. (2013)[248]	14 normal weight adults	Subjects were exposed to flavors either paired with calories or not paired with calories. After these training sessions, the subjects were scanned. During the scan they were given a tasteless solution, the flavor with calories, the flavor with no calories, and a control flavor.	Participants preferred flavors paired with calories. Nucleus accumbens and hypothalamic response was associated with circulating glucose and not liking. Insula response was related to liking the caloric containing flavor.
Jastreboff A, et al. (2013)[170]	50 lean and obese adults	Fasting insulin levels were assessed. Subjects were scanned using an individualized stress and food cue paradigm.	Obese subjects showed increased BOLD response in the striatal, insula, and hypothalamic regions during food cues and stress cues compared to the lean. Food craving, insulin, and HOMA-IR, positively correlated with activity in the cortico-limbic-striatal brain regions during food and stress cues in the obese group. HOMA-IR and food craving was mediated by striatum, insula, and thalamus in the obese group.
Sun X, et al. (2015)[249]	32 healthy adults	Subjects were scanned using the milkshake paradigm. Subjects were scanned fasted and fed. Blood draws were taken in the scanner as were perceptual ratings. After the scan the subjects were given an ad libitum meal.	Amygdala BOLD response to the taste of milkshake when stated (but not when hungry) predicted weight change in subjects without Taq1A allele. Subjects with the Taq1A allele showed a positive association between the caudate and weight change. When satiated gustatory input from the amygdala to the hypothalamus is one way, when hungry, the relationship between the hypothalamus and amygdala is bidirectional.

Continued Table 2.1 Nolan-Poupart S, et al. (2013) [250]	23 healthy adults	Milkshake paradigm was used during the scan followed by an ad libitum milkshake challenge.	BOLD response in the periaqueductal gray region was associated with ad libitum milkshake consumption.
Cosgrove K, et al. (2015) [251]	29 healthy adults	PET scan and fMRI with a milkshake paradigm.	Decreased dorsal striatal response to milkshake and BMI. BMI was positively associated with dopamine receptor 2 and 3 availability.
Small D, et al. (2003)[252]	7 healthy adults	PET scan in fed and fasted state. Given favorite meal and asked to rate pleasantness.	Increased dopamine release in the dorsal putamen and caudate in the fasted condition. Amount of dopamine released was correlated with meal pleasantness.
Stice E, et al. (2008) [253]	33 adolescent girls	Scanned with the milkshake paradigm.	Obese compared to lean showed increased BOLD response in the gustatory cortex, somatosensory regions in anticipation. In response to intake, obese subjects showed decreased BOLD response in the caudate.
Babbs KR, et al. (2013) [254]	25 adults, 12 normal weight and 13 overweight or obese	Scanned with the milkshake paradigm (2 milkshake flavors to prevent taste habituation) and a tasteless solution. Impulsivity was also assessed with a go/no go task outside the scanner.	Negative association between BMI and BOLD response to the milkshake in the caudate, positive association seen in the putamen. In the overweight subjects, impulsivity was associated with caudate response to milkshake. Relationship between BMI and caudate is mediated by impulsivity and not food reward.
Chouinard-Decorte F, et al. (2010) [255]	26 adults, 13 normal weight and 13 overweight or obese	Scanned with the milkshake paradigm both taste and smell cues	Greater amygdala BOLD response to milkshake taste and smell cues compared to the normal weight. Hunger was associated with amygdala response to the milkshake taste in the healthy weight group. Amygdala response to food aromas predicted weight gain one year later.
Stice E, et al. (2015) [256]	153 adolescents mean BMI 20.8	Screened for Taq1A polymorphism and scanned with a milkshake paradigm and a monetary paradigm.	Lower BOLD response in the caudate correlated with BMI in those with the Taq1A allele. Increased OFC to anticipation of milkshake predicted future body fat.
Stice E, et al. (2013) [257]	34 healthy adolescents	Scanned during a food picture paradigm and milkshake receipt paradigm	Caloric deprivation was positively correlated with the anterior cingulate cortex, orbitofrontal cortex, putamen, and precentral gyrus.
Stice E, et al. (2013) [258]	106 lean adolescents	Scanned with a variety of taste cues with varying fat and sugar content: high fat-	High fat was related to greater BOLD response in caudate, postcentral gyrus, hippocampus, and inferior

Continued Table 2.1		high sugar, high fat-low sugar, low fat-low sugar, low far-low sugar.	frontal gyrus. High sugar was associated with BOLD response in the insula, putamen, Rolandic operculum, and thalamus.
Burger KS, et al. (2014) [259]	27 adolescents mean BMI 23	Milkshake taste, cola taste, and cola advertisement paradigms were used during scanning.	Receipt and anticipation of cola was associated with BOLD response in regions related to gustatory, oral somatosensory, and reward processing. Advertisements were associated with increased BOLD response in gustatory regions and visual regions. Habitual cola consumers compared to non-consumers showed increased BOLD response in the posterior cingulate gyrus to cola logos.
Burger KS, et al. (2014) [260]	25 female adolescents mean BMI 24	Milkshake paradigm was used during the scan and body composition was assessed after a two-year follow up.	Increased BOLD response in the caudate to cue predicting milkshake receipt over repeated exposure. Over repeated exposure there was a decrease in BOLD response in the putamen and in the ventral pallidum. Those who showed the highest increases in ventral pallidum and greatest decrease in caudate also had higher BMIs at follow up.
Page K, et al. (2013) [77]	20 healthy adults	Two scanning sessions with either a fructose or glucose drink preload.	Greater reduction in CBF after glucose compared to fructose in the hypothalamus. Glucose was associated with increased functional connectivity compared to baseline between the hypothalamus, thalamus, and striatum. Fructose was related to increased connectivity between the hypothalamus and thalamus.
Page K, et al. (2011) [261]	21 healthy subjects	Subjects were scanned with a hyperinsulinemic clamp. During the scan subjects were shown pictures of foods and asked to rate the foods.	Hypoglycemia is associated with CBF in limbic-striatal regions while looking at food cues. Euglycemia was associated with the medial prefrontal cortex, and less interest in food stimuli.
Adam TC, et al. (2013) [235]	12 overweight Hispanic girls (8-11 y)	Frequently sampled intravenous glucose tolerance test and fMRI scan with a food image paradigm showing high and low calorie foods.	Insulin sensitivity was inversely associated with BOLD response in the anterior cingulate, insula, OFC, and Rolandic operculum when looking at high calorie foods compared to low calorie foods. When waist circumference was included in the model, results were attenuated.
Martens MJI, et al. (2013)[262]	40 adults, 20 normal weight	Two fMRI scans, one fasted and the other after a	The overweight subjects exhibited decreased inhibitory control. The

Continued Table 2.1	and 20 overweight	mixed macronutrient breakfast. The fMRI paradigm showed pictures of foods and non-food items. Subjects also completed an impulsivity task.	overweight subjects had higher BOLD response in the anterior cingulate cortex in the fasted state in response to the food pictures, and lower BOLD response in the fed state compared to the lean. Additionally, in the fed state the lean subjects had higher BOLD response in the prefrontal cortex to food pictures compared to the overweight.
Luo S, et al. (2013) [263]	13 Hispanic women BMI range 28-40	Subjects were shown pictures of food and non-food items.	Foods compared to non-foods were associated with BOLD response in the ORF, vmPFC, anterior cingulate, insula, nucleus accumbens, amygdala, hippocampus, and occipital cortex. High calorie foods compared to non-foods were associated with BOLD response in the insula, OFC, PFC, anterior cingulate cortex, amygdala, and right striatum. Hunger ratings were higher after seeing pictures of high calorie foods. Striatal BOLD response when viewing high calorie food images was correlated with waist circumference.
Tryon M, et al. (2015) [237]	19 adult overweight women	The participants were given sucrose or aspartame sweetened beverages to drink 3 times daily for 2 weeks. Salivary cortisol was collected and participants were scanned while preforming the Trier Social Stress Task.	Sucrose consumption, compared to aspartame, was associated with BOLD response in the hippocampus during the stress task and with stress induced cortisol response.
Jastreboff A, et al. (2014) [264]	40 adolescents, 25 obese and 15 normal weight	Scanned with a high calorie food, low calorie food, and nonfood visual stimuli paradigm.	In the striatal-limbic regions, obese compared to lean subjects showed increased BOLD response to high calorie foods compared to nonfood cues. Higher leptin concentrations were correlated with increased BOLD response to high calorie foods in all subjects.
Jastreboff A, et al. (2016) [234]	38 adolescents, 14 lean and 24 obese	Subjects drank either a fructose or sucrose drink and then were scanned using perfusion methods.	After the glucose drink, obese subjects showed decreased CBF in the PFC and increased CBF to the hypothalamus. Ghrelin and insulin after both drinks were associated with increased CBF in the hypothalamus, thalamus, and hippocampus in the obese compared

Continued Table 2.1			to lean. In all subjects there was increased CBF in the striatum after the fructose drink compared to the glucose drink. Obese compared to lean subjects also exhibited reduced connectivity between executive, homeostatic, and hedonic brain regions.
Valetin VV, et al. (2007)[265]	19 adults BMI not reported	Subjects were scanned with a paradigm to test incentive salience between tomato juice, orange juice, and milkshake. Post scanning subjects were fed one taste to devalue the flavor. The subjects were scanned again in the devalued state.	BOLD response in the OFC was highly responsive during selection of taste in the scanning paradigm. With high activity in the devalued state compared to the normal value state.
Tricomi E, et al. (2009)[266]	32 adults BMI not reported	Subjects were scanned with a free-operant task where fractal cues were rewarded on a variable interval, which were consumed after the scans.	BOLD response in increased to task sensitivity in the right putamen as the training progressed. Suggesting a shift from goal-directed learning to habit based learning.
Volkow ND, et al. (2009)[267]	21 adults with BMIs ranging from obese to lean	Subjects were scanned using PET while doing a cognitive task or resting.	There was a negative correlation between BMI and metabolic activity in the prefrontal cortex and cingulate gyrus.
Nummenmaa L, er al. (2012)[268]	25 subjects, 19 morbidly obese and 16 normal weight adults	Subjects were scanned using PET during a euglycemic hyperinsulinemia clamp and an fMRI. During which subjects were shown pictures of foods.	Glucose uptake was higher in the dorsal caudate in the obese compared to lean. During the fMRI and viewing the food pictures, BOLD response in the caudate was higher in the obese than the lean.
McClure S, et al. (2003)[269]	28 adults BMI not reported	Subjects were scanned and performed a passive prediction error juice paradigm where they saw a cue (light) and then got a taste of juice. There was a variable amount of time between the cue and the taste.	Reward prediction error correlated with BOLD response in the striatum.
Born JM, et al. (2011)[270]	15 normal weight adults	Scanned twice, once fasted once satiated. Subjects completed a food choice paradigm. The paradigm discriminated between liking and wanting.	Dietary restraint was related to liking in the amygdala, striatum, thalamus, and cingulate cortex. In the hypothalamus and striatum, BMI and hunger predicted wanting. Wanting in the striatum predicted energy intake.

Continued Table 2.1 Demos K, et al. (2011)[271]	100 adults, 50 chronic dieters and 50 non-dieters (were they overweight/obese)	All subjects consumed either a water preload or a milkshake preload. They were then imaged while viewing food and non-food cues.	Non-dieters compared to dieters showed increased striatal activity to appetizing food when they had water preload compared to the milkshake preload.
Siep N, et al. (2009)[272]	12 normal weight adults	Subjects were scanned twice with a food image paradigm in the fasted and fed state.	Fed female subjects exhibited increased reward processing in response to low calorie foods. Fasted state increased BOLD response in reward processing areas to high calorie foods.
LaBar KS, et al. (2001)[273]	17 adults BMI not reported	Subjects viewed images of food and non-food objects in hungry and satiety conditions.	Food images were related to increased BOLD response in the amygdala, parahippocampal gyrus, and anterior fusiform gyrus.
Arana FS, et al. (2003)[274]	12 male adults BMI not reported	Subjects underwent a PET scan. They preformed a restaurant task, where they read a menu and selected the items they would like to eat.	Activity in the amygdala was active when viewing palatable food items on the menu. The OFC was active during incentive judgment and goal selection portions of the task.
Tataranni PA, et al. (1999)[275]	11 normal weight adult males	Subjects were scanned in the fasted and fed states using PET.	Hunger was associated with CBF in the hypothalamus, insula, OFC, anterior cingulate cortex, and parahippocampal formations. Satiation was associated with increased CBF in the prefrontal cortex and parietal lobule. Insulin concentrations were negatively correlated with CBF in the insula and OFC.
Pelchat ML, et al. (2004)[276]	20 adults, 10 on monotonous diet and 10 on normal diet	Subjects were scanned and were asked to imagine their favorite foods at certain cues.	The monotonous diet was associated with increased cravings for favorite foods. BOLD response in the hippocampus, insula, and caudate were associated craving during the food cues.
Karhunen L, et al. (1999)[277]	22 adults, 10 obese and 12 normal weight	Subjects were scanned using PET. During the PET scan they viewed either a landscape or pictures of food. Blood was drawn 5 times.	Inverse relationships between CBF in the hypothalamus and leptin during food images in the obese.
Gordon CM, et al. (2000)[278]	8 normal weight adults	Subjects were scanned using PET. They were shown images of high caloric, low caloric and non-food items and asked to indicate hunger on	Desire to eat was inversely associated with CBF in the left insular cortex when viewing high calorie compared to low calorie foods.

visual analogue scales.

All scanning is referring to functional magnetic resonance imaging unless otherwise noted. OFC=orbital frontal cortex; CBF=cerebral blood flow; PET= positron emission tomography; BOLD= blood oxygenation level dependent; vmPFC=ventromedial prefrontal cortex.

In conclusion, hunger and the motivation to eat is more than the homeostatic need to balance one's blood sugar. The act of consuming food is rooted in both hedonic and homeostatic mechanisms, and therefore both systems must be examined. Additionally, obesity can be viewed as the dysregulation of both the homeostatic and hedonic pathways, and therefore research is needed to examine how both pathways can be manipulated to ameliorate or prevent obesity and related metabolic dysfunction.

This project proposed to examine SSB intake as modifiable element in both homeostatic and hedonic mechanism of perceived hunger. **The aims of this project were as follows: 1a**: To examine and compare how exposure to a high/low sugar breakfast and lunch influences metabolic biomarkers (PYY and ghrelin) between frequent and naïve sugar consuming groups in NHB and Hispanic adolescents (14-17 y). **1b**: To examine and compare how high/low sugar meals influence subsequent ad libitum dietary intake by SSB intake. **2a**: To examine the association between SSB intake and adiposity depots (i.e., visceral and hepatic fat) in Black and Hispanic adolescents (14-17 y). **2b**. To examine the relationship between habitual SSB intake and cortisol response, independent of adiposity depots, in Black and Hispanic youth. **3a.** To examine and compare the effect of free-living dietary intake, ad libitum test intake, and metabolic biomarkers on hunger, and satiety. **3b.** To explore how reward pathway response to prediction error is correlated with SSB intake in obese and overweight Hispanic children (7-10 y).

Chapter3: The impact of sugar sweetened beverage intake on hunger and satiety in minority adolescents.

Shearrer GE, O'Reilly GA, Belcher BR, Daniels MJ, Goran MI, Spruijt-Metz D, Davis JN. Appetite. 2016

GES was responsible for data analysis and manuscript preparation.

ABSTRACT

Background: Limited research has examined the effects of habitual SSB consumption on hunger/fullness ratings and gut hormones. This study hypothesized that high versus low intakes of habitual SSBs would result in greater hunger, decreased fullness, and a blunted gut hormone response, however the high versus low fiber group would exhibit decreased hunger and increased fullness.

Methods: This was a randomized crossover feeding trial with 47 African American and Hispanic adolescents. The experiment included at least 24-hour recalls to assess free-living dietary intake. During the test meal phase, subjects were served breakfast and lunch. During the ad libitum meal phase, subjects were fed an ad libitum dinner. During the test meal phase, blood was drawn every 30 minutes for 3 hours. During the ad libitum meal phase, hunger and fullness visual analogue scales were completed. For this analysis, subjects were grouped into the following habitual SSB categories: low SSB (≤1 SSB serv/day), medium SSB (>1 - <2 serv/day), and high SSB (≥2 serv/day). Fiber categories were created based on quartiles of intake. Mixed modeling was used to explore how SSB and fiber categories predicted ghrelin/PYY values and hunger/fullness ratings across time within and between test meals. The following a priori covariates included: sex, ethnicity,

age, and obesity status.

Results: The low SSB group had higher fullness ratings over the ad libitum meal compared to the high SSB group (β =-0.49, CI=(-0.89, -0.08), p=0.02) and higher ghrelin concentrations than the medium and high SSB group over the test meal phase (β =-1.86, CI=(-2.81, -0.92), p<0.01).

Discussion: Habitual SSB intake appears to play a key role in moderating fullness responses possibly via ghrelin.

INTRODUCTION

NHB and Hispanic adolescents are disproportionately at risk for obesity and metabolic disease [279], with 39% and 38% being obese, respectively, compared with 31% of NHW adolescents in the United States [5]. Additionally, adolescence is a time of increasing autonomy when youth begin to make independent dietary choices, and is therefore an ideal time to improve dietary habits in a high risk population [98].

The increased prevalence of obesity in adolescent minority populations in the US can be attributed to a multitude of factors. Two modifiable factors are the high intake of added sugars and the low intake of dietary fiber. A major contributor to added sugar in the diet of adolescents is SSB such as carbonated sodas, sports drinks, and fruit drinks [58]. NHB and Hispanic youth consume on average more calories from SSB than their NHW peers [58]. Increased SSB intake in adolescents was associated with higher systolic blood pressure, a risk factor for hypertension and cardiovascular disease [131]. Additionally, SSB intake has been associated with increased body weight in adolescents [117–119], with a dose

responsive relationship between SSB intake and obesity risk in adolescents who consume four or more eight ounce servings per week [120]. Longitudinal data has shown that a single serving of SSB per day at age 15 years increased BMI and waist circumference over six years [116].

Adolescents have been found to consume more fructose than any other age group over the last 30 years, and it has been suggested this in large part is due to use of HFCS [136]. The principal sweetener in SSB is HFCS, which is comprised of nearly 50% more fructose than glucose [72]. Total dietary fructose has been shown to be positively related to increased visceral adiposity, increased blood pressure, and HOMA-IR in a large sample of adolescents [137]. An experiment comparing glucose, HFCS, sucrose, and sucralose found that food intake after a HFCS or sucrose drink led to higher energy intake at a subsequent ad libitum meal [139].

Not only are adolescents more likely to consume fructose, but also adolescents are less likely to consume high fiber foods compared to other age groups. Adolescents consume on average 14 grams of fiber per day, substantially below the recommended 25 to 30 grams per day [280]. Increasing satiety post meal is a desirable means of controlling and reducing weight. Satiety can be qualitatively measured via visual analogue scales and predicted via metabolic hormones such as PYY and ghrelin [281]. Increased dietary fiber is an attractive means to increase post meal satiety as it is easily substituted in the diet and has been associated with weight loss [282]. However current research is inconclusive regarding the effectiveness of fiber to improve satiety [283,284]. Preliminary research

examining the difference between the effect of high sugar versus high fiber meals on satiety measures has shown mixed results [285,286].

Previous research has focused on the satiating action of acute intake of either fiber or SSB [287–291]. Research has shown acute satiety after a sugar pre-load [292], however the effect of prolonged sugar consumption is, to date, understudied. An acute administration of a sugary beverage was associated with increased short term appetite in boys 9 to 14 years [139]. Additionally, Cassady and colleagues has suggested lower satiety after a caloric liquid compared to an isocaloric solid, possibly due to a decreased gastric distention in the liquid as well as an aberrant endocrine response [293]. Aberrant endocrine response is a hallmark of homeostatic break down over time, such as leptin resistance and ghrelin suppression in obesity [32,294]. These acute studies do not take into account the effect of habitual, free-living, intake of fiber and SSB. Given the mixed results of high sugar versus high fiber intake on satiety and the lack of research in minority youth, this study sought to compare the effects of free-living SSB intake and fiber intake on ghrelin and PYY levels during an acute fiber and sugar test meal challenge, and perceived hunger and fullness after the meal challenge during an ad libitum meal. Along these lines, we hypothesized that free-living high SSB consumers compared to low SSB consumers would have lower ghrelin and PYY regardless of the test meal and higher hunger and lower fullness during the ad libitum meals. We also hypothesized that free-living low fiber consumers compared to high fiber consumers would exhibit lower serum ghrelin and PYY regardless of the test meal type and be more hungry and less full during the ad libitum meals.

SUBJECTS AND METHODS

Data comes from the FAME crossover feeding trial at USC. Conclusions based on the main outcomes and a detailed methodology can be found elsewhere [295]. This study was conducted at the USC Health Sciences campus in Los Angeles, California from 2008 to 2011. Subjects were recruited from hospitals, clinics, churches, schools, and community center around the Los Angeles area between 2007-2010. Hispanic and NHB youth, ages 14 to 17 years, with a BMI above the 85th percentile for age were recruited into the study. Subjects were excluded if they exhibited evidence of diabetes, were currently in a weight loss program, or used medications that influenced insulin or body composition. Informed written parental consent and participant assent was acquired before all testing procedures. The Institutional Review Board of USC approved all study procedures.

Baseline measures

An initial, separate, baseline visit gathered demographic data and a licensed pediatric medical care provider collected body composition data and performed a physical exam. After the baseline visit, subjects were called for three multiple pass 24-hour dietary recalls by trained interviewers using the Nutrient Data System for Research (NDS-R 2010, University of Minnesota, Minneapolis, MN). All diet recalls were performed within one month of the first testing visit.

Testing

The testing visits were each nine hours long. The order in which subjects received either a high sugar and low fiber breakfast and lunch or a low sugar high fiber breakfast and lunch was randomly assigned. All participants completed both meals. Details pertaining to the meal type can be found here [295]. Subjects arrived at the USC health sciences center at 7:00am, where they completed anthropometrics, blood pressure testing, and questionnaires. The first blood draw was at 8:00 am. The breakfast was served directly after the initial blood draw and the lunch was served at noon. Subjects were given 15 minutes to finish the meals. The final blood draw occurred at 1:00 pm. From 1:00 pm to 4:00 pm the subjects were allowed access to an ad libitum meal tray and were instructed that they could eat as much or as little as they desired. A one-way mirror separated subjects and researchers so that the subject could not see the researchers. The subject also had access to a variety of games (Wii Fit, Dance Dance Revolution), movies, television, books, a craft corner and treadmill for the entire study period. After a two to four week washout period, subjects returned for the second identical testing visit but with the alternate test meal.

Appetite/Satiety measurement

A Registered Nurse placed a saline lock intravenous catheter in the antecubital vein of the non-dominant arm. Blood was drawn every 30 minutes during the test meal phase of the visit (the first five hours). Although blood was drawn on all subjects, funding only allowed a random subset of subjects to be assayed for PYY and ghrelin.

Every 30 minutes for the entire lab visit the subject was given a 100mm-visual analogue scale (VAS) to rate how hungry he or she felt, as well as a VAS to rate how full he or she felt. All blood collected was centrifuged and processed within one hour of collection and was stored at -70 until analysis. Active ghrelin concentrations were analyzed via enzyme-linked immunosorbent assay (ELISA) (Millipore, Darmstadt, Germany). Total PYY was analyzed by radioimmunoassay (RIA) (Millipore, Darmstadt, Germany).

Subjects were classified for high volume SSB consumers based on the dietary recall data (**Tables 3.1 and 3.2**): low SSB consumers (≤ 1 servings per day), medium SSB consumers (>1 - ≤ 2 servings per day), and high SSB consumers (>2 servings per day). SSB consumer groups were used evaluate the change in dependent variables in a dose dependent manner, as well as to minimize reporter bias. Drinks considered SSB were as follows: sweetened soft drinks, sweetened fruit drinks, sweetened tea, sweetened coffee, sweetened coffee substitutes, and sweetened water. A serving was defined as 8 fluid ounces. Fiber was divided into the following quartile categories for test meal analysis based on dietary recall data: very low (≤ 3.6 grams per day), low (between 3.6 and 9.9 grams per day), medium (between 9.9 and 13.5 per day), high volume consumers (>13.5 grams per day). For the ad libitum meal analysis fiber was divided into the following quartile categories based on dietary recall data: very low fiber intake (≤ 5.8 grams per day), low fiber intake (>5.8 - ≤ 9.8 grams per day), medium fiber intake (>9.8 - ≤ 15.5 grams per day), high fiber intake (>15 grams per day). Because of the difference in sample sizes between the test meals phase and the ad libitum meal phase two different quartiles were calculated for each phase. Hunger and fullness ratings, which ranged from

0 to 100 were transformed using a logit function to better satisfy the normality assumption in the models. Of the appetite variables, hunger and fullness were analyzed from 300 to 480 minutes after 8:00 am (during the ad libitum meal period), while ghrelin and PYY were analyzed from 0 to 300 minutes, starting at 8:00am (during the test meal phase). Differences in hunger, fullness, ghrelin, and PYY concentration were evaluated initially between the two test meal types. No differences between the high sugar and low fiber and the low sugar and high fiber meal were found. Therefore, the data from both test meals was used in the evaluation of hunger, fullness, ghrelin, and PYY concentration.

Statistical Analysis

All analyses were performed with R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Initially, the high sugar and low fiber breakfast and lunch were compared to the low sugar high fiber test meals using a repeated measure t-test. The LME function from the nlme 3.1-119 package was used to fit linear mixed effects models with a random intercept and slope to assess the effect of meal type, SSB group, fiber group, and time on the two appetite variables (hunger, fullness). In the model, the fixed effect of time was modeled using a spline with three degrees of freedom with the basis matrix for natural cubic splines (ns) function from the splines 3.1.2 package in R. The baseline models included the dependent variable of interest (hunger, fullness, ghrelin or PYY concentration) and only the spline for time as the independent variable. The following other independent variables were added stepwise: SSB category, fiber category, ethnicity, overweight or obese status, sex, and energy intake (calories per day).

Multiplicative interaction terms were added to the models according to individual independent variable significance also in a stepwise fashion. Log-likelihood tests were used to compare the relative fit of nested models after each stepwise categorical variable addition with the lrtest function from the lmtest 0.99-33 package. Statistical significance was set at $p \leq 0.05$ and beta value (β) refers to the regression coefficient.

RESULTS

Participant Characteristics

A total of 93 subjects were randomized into the initial meal study. Seven withdrew after randomization for a total of 87 participants that completed both meals. Of the 87 who completed both study visits, 47 participants had two or more diet recalls within one month of each other and test visit data. A subsample of 18 subjects, concentrations for which assayed blood samples were available, was analyzed for ghrelin and PYY. There were no significant differences in sex, caloric intake, overweight or obese status between the subsample which was assayed for PYY and ghrelin and the overall sample, although the blood subsamples were made up predominately of Hispanic youth (only 7.7% NHB) and all NHB subjects were medium SSB volume consumers. **Tables 3.1 and 3.2** summarize the demographics of the entire sample (the ad libitum meal phase) and the subset (the test meal phase). Neither SSB nor fiber intake were significantly different by day of recall. None of the variables of interest were different between high sugar and low fiber nor high fiber low sugar test meals.

Table 3.1: Demographics by sugar sweetened beverage

			SSB Groups	
Demographic	Total	High	Medium	Low
Test meals phase	N=18	N=4	N=7	N=7
Age (years)	16.2 ±1.0	16.7±0.7	16.6±1.1	16.0±0.9
Ethnicity (Hispanic)	92.2%	92.2%	100%	100%
Sex (male)	76%	82.6%	100%	62.1%
BMI	32.2±7.2	31.6±9.0	37.7±5.4	29.8±5.2
Energy intake (kcal/d)	922.2±3	709.2±230	1405.2±269	791.7±475
Fiber intake (g/d)	12.6±5.6	12.5±2.2	15.9±6.2	10.9±5.6
Total sugar intake (g/d)	98.4±45.5	143.6±46.8	115.2±32.9	69.8±24.5
Ad libitum meal phase	N= 47	N=8	N=18	N=21
Age (years)	16.6 ±1.4	16.8 ±1.1	16.5±1.5	16.1 ±1.0
Sex (male)	46.9%	50%	57%	40%
Ethnicity (NHB)	27.8%	35.7%	28.5%	25%
BMI	32.9 ±6.2	33.5±7.7	34.9±5.3	30.6±5.9
Energy intake (kcal/d)	1856.4 ±701	2259.5±632	1895.9±783	1518.4±565
Fiber intake (g/d)	12.3 ±5.2	14.4 ±4.9	11.9 ±5.8	12.2 ±5.8
Total sugar intake (g/d)	103.8±49.2	131.6±42.9	100.0±32.7	79.8±39.7

All data is presented as mean ±standard deviation

Table 3.2: Demographics by fiber consumers

		Fiber Groups		
Demographic	High	Medium	Low	Very Low
Test meals phase	N=5	N=4	N=4	N=4
Age (years)	17.0±0.8	16.1±0.9	16.5±0.5	15.2±0.7
Ethnicity (Hispanic)	100%	92.2%	100%	100%
Sex (male)	77.7%	100%	65.4%	41.2%
BMI	34.4±8.2	30.7±7.0	31.1±3.6	31.2±5.9
Energy intake (kcal/d)	1136.7±277	1135.3±42	430.6±268	467.1±379
Fiber intake (g/d)	18.6±3.9	11.2±0.8	9.1±0.3	5.5±1.9
Total sugar intake (g/d)	108.7±58.4	116.2±24.4	69±33.3	67.3±19.9
Ad libitum meal phase	N=14	N=13	N=14	
Age (years)	16.6±0.9	16.2±1.4	16.5±1.1	
Sex (male)	57%	46.1%	50%	
Ethnicity (NHB)	27.2%	23.0%	50%	
BMI	35.5±7.5	30.0±4.9	31.4±6.6	
Energy intake (kcal/d)	1317.9±553	1034.1±355	827.9±485	
Fiber intake (g/d)	19.5±3.4	11.3±1.3	7.7±1.8	
Total sugar intake (g/d)	121.8±55.0	107.5±32.0	74.2±33.0	

All data is presented as mean ±standard deviation

Test meal phase

The effect of SSB was significant in the ghrelin model (p=0.03). In particular, the low SSB consumers exhibited higher ghrelin concentrations compared to the high SSB consumers over time during the test meal (β =-1.86, CI=(-2.81, -0.92), p<0.01). This difference in SSB consumption is visualized in **Figure 3.1** as the difference in the shift of the SSB group curves. There were not significant differences between fiber groups and serum ghrelin concentrations over time during the test meal.

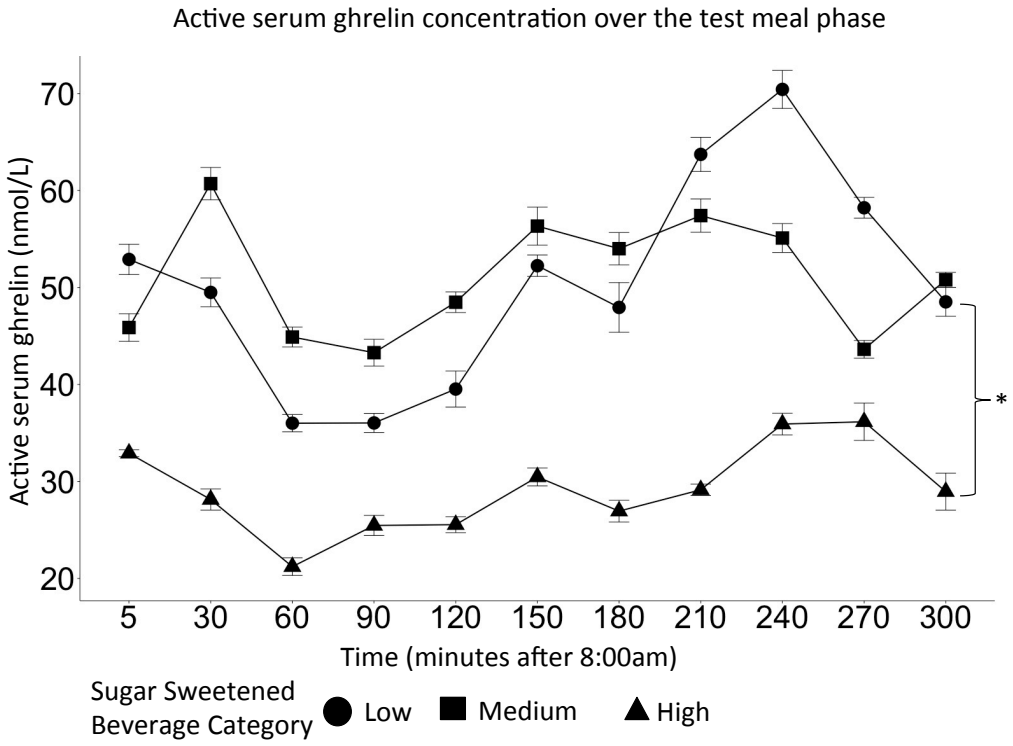

Figure 3.1: Serum ghrelin concentration over time during the test meals phase. This sample contained 18 subjects. High SSB consumer group exhibited lower active ghrelin than the low SSB group (p<0.01). Error bars represent the confidence interval.

There were no significant differences between SSB groups and serum PYY over time during the test meal. Nor were there significant differences between fiber groups and serum PYY concentrations over time during the test meal.

Ad libitum meal phase

There were no significant differences between SSB groups and hunger ratings during the ad libitum meal phase. Nor were there significant differences between fiber groups and hunger ratings during the ad libitum meal phase.

SSB categories significantly improved the predictive ability of the fullness model compared to baseline ($p=0.03$). The low SSB group was more full compared to the high SSB group during the ad libitum meal phase (β =-0.49, CI=(-0.89, -0.08), $p=0.02$). This difference in SSB consumption is visualized in **Figure 3.2** as the difference in the shift of the SSB group curves. There were no significant differences between fiber groups and fullness ratings during the ad libitum meal.

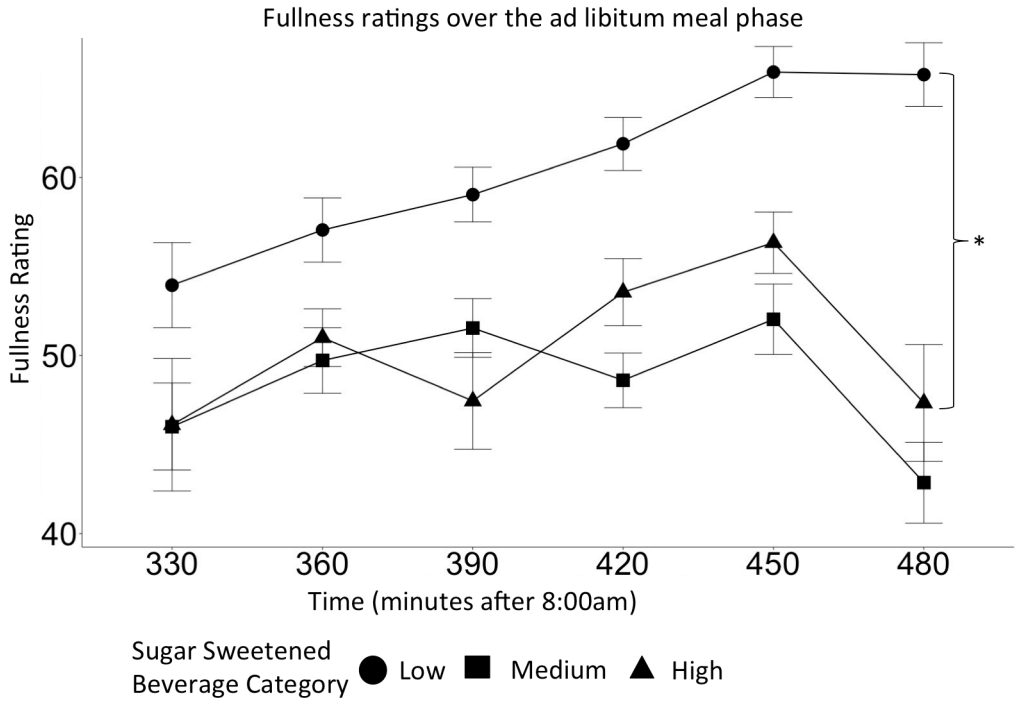

Figure 3.2: Fullness ratings as indicated by visual analogue scale over the ad libitum meal between fiber intake groups. The low SSB consumers, compared to the high SSB consumers, where more full (p=0.02). Error bars represent the confidence interval.

DISCUSSION

This is the first study to show that free-living SSB intake influences feelings of fullness and ghrelin response during a crossover meal design. Although initially there were no differences between the two meals given during the crossover meal trial, free living SSB intake revealed differences overall. High SSB consumers were on average more full during the ad libitum meal phase compared to low SSB consumers. Interestingly, SSB consumption did not influence hunger over the ad libitum meal phase.

Low SSB consumers exhibited higher ghrelin concentrations during the test meals phase compared to high SSB consumers. However, SSB group did not influence PYY. Dietary fiber was not significantly related to hunger, fullness, ghrelin or PYY.

Previous research has focused on acute intake of SSB or dietary fiber on appetite [139,287,288,290,291]. Studies focusing on immediate satiety after a sugar preload have found an increase in satiety and caloric compensation at ad libitum meals, which aligns with the well accepted glucostatic theory [296,297]. The glucostatic theory postulates that the increase in blood glucose and blood insulin acts as a satiety signal in the short term and allows for caloric compensation. This current study suggests an alternative hypothesis for free-living SSB consumption, suggesting high SSB intake is associated with lower feelings of satiety. Preceding work has suggested that SSB increases caloric intake, greater than that achieved through SSB intake alone, and therefore SSB may suppress satiety or increase appetite [144]. SSB intake has also been associated with weight gain, however when caloric intake was included in the models the relationship was attenuated [118,298]. Since this current study controls for caloric intake and weight status, the findings further suggest that the relationship between SSB and fullness are independent of caloric intake and BMI in this sample.

Furthermore, this study's examination of metabolic hormones further reveals a possible mechanism. The glucostatic theory, mentioned above, breaks down over time in overweight and obese subjects resulting in hyperinsulinemia and eventually insulin resistance. Emerging research shows a similar pattern with leptin resistance [294] and ghrelin suppression in obesity [32]. The HELENA study showed that SSB consumption was

positively related to homeostasis model assessment-insulin resistance index (HOMA-IR) in European adolescents [133]. Insulin, and in particular HOMA-IR, has been shown to be negatively associated with serum ghrelin [184]. This study found suppressed ghrelin in the highest SSB consumer group, independent of weight status. The association between HOMA-IR and SSB intake and these results showing suppressed ghrelin for high SSB consumption suggests a possible mechanism for long term alterations in fullness. High SSB consumption appears to suppress ghrelin resulting in decreased fullness ratings in the high SSB group. Further research is needed to explore the relationship between satiety, SSB, and other endocrine signals such as insulin and leptin, which have been shown to influence ghrelin.

There are several limitations of the current study. The relatively small sample size (n=47) is a limitation, particularly the small sample (n=18) with PYY and ghrelin data; thus lack of statistical significance could be due to low power. In addition, this study included more Hispanic children than NHB children; therefore, these results may not be applicable for the general NHB population. Additionally, this study was in overweight and obese Hispanic and NHB adolescents, and the findings may not be generalizable to other ethnicities and/or lean individuals. In addition, since this study is a crossover meal test study and cross-sectional in nature, causality of SSB intake on satiety status and ghrelin concentration cannot be assessed. Finally, it should be noted that only two or three dietary recalls were performed, which could impact the reliability of the measure. Ideally, four to five dietary recalls would provide a more robust measurement of habitual

dietary intake [299]. Furthermore, the 24-hour recall method is subject to reporter bias, and may under estimate actual intake.

In conclusion, high free-living SSB intake appears to play a role in moderating fullness in a meal test. These findings highlight that future research should consider the effects of long term free-living diet, particularly SSB consumption. Additionally, high intake of SSB appears to lower ghrelin response. Future interventions should focus on reducing SSB consumption.

Chapter 4: Associations among sugar sweetened beverage intake, visceral fat, and cortisol awakening response in minority youth

Shearrer GE, Daniels MJ, Toledo-Corral CM, Weigensberg MJ, Spruijt-Metz D, Davis JN. Physiology and Behavior. 2016

GES was responsible for data analysis and manuscript preparation.

ABSTRACT

Background: Abdominal adiposity has long been associated with psychosocial stress and associated cortisol dysfunction. However, the relationship of sugar-sweetened beverage (SSB) intake specifically with cortisol variability and visceral adipose tissue (VAT) is unknown. The objective of this study was to examine the relationships between SSB intake, VAT, and cortisol response in minority youth.

Methods: In this cross sectional study, 60 overweight/obese NHB and Hispanic adolescents ages 14-18 years underwent a Magnetic Resonance Imaging (MRI) scan to assess VAT. Cortisol awakening response (CAR) was measured via multiple salivary samples, and SSB intake via multiple 24-hour diet recalls. SSB intake was divided into the following: low SSB consumers (< 1 servings per day), medium SSB consumers (≥1 - <2 servings per day), high SSB consumers (≥2 servings per day). Analysis of covariance were run with VAT and CAR as dependent variables and SSB intake categories (independent variable) with the following a priori covariates: sex, Tanner stage, ethnicity, caloric intake, and body mass index.

Results: The high SSB intake group exhibited a 7% higher VAT compared to the low SSB intake group (β=0.25, CI:(0.03, 0.33), p=0.02). CAR was associated with VAT

(β=0.31, CI:(0.01,0.23), p=0.02). The high SSB intake group exhibited 22% higher CAR compared to the low SSB intake group (β=0.30, CI:(0.02,0.48), p=0.04).

Conclusion: This is the first study exploring the relationship between SSB, VAT, and CAR. SSB consumption appears to be independently associated greater abdominal adiposity and higher morning cortisol response in overweight and obese minority youth. This study highlights potential targets for interventions specifically to reduce SSB intake in a minority youth population.

INTRODUCTION

Adolescence is a key time to address obesegenic behaviors as children transition into adulthood and begin to make independent dietary choices[98]. Adolescent weight gain has been shown to be persistent over the life cycle, overweight teens are four times more likely to become overweight or obese adults than normal weight peers [300]. Increasingly, the role of SSB consumption has been highlighted as a modifying factor for overweight and obese adolescents. SSB intake has been shown to cluster with multiple other unhealthy lifestyle and eating behaviors in adolescents, such as: intake of fried foods, desserts, increased sedentary activities, and decreases in fruit and vegetable intakes [123]. A recent meta analysis indicated a strong association between SSB consumption and risk of becoming obese in children [53]. In general, high school students are more likely to consume SSB compared to middle and elementary school children [100]. Moreover, NHB and Hispanic high school students consume more carbonated and non-carbonated SSBs respectively than non-Hispanic white high school students [100]. This coincides with NHB

and Hispanic minorities also being disproportionately at risk for obesity, with approximately 1 in 3 NHB and Hispanic youth (12-19 years) being overweight or obese [5].

Independent of BMI, SSB intake has been implicated as a determinant of type 2 diabetes, and metabolic syndrome [55], however SSB intake is one of many factors associated to metabolic risk. VAT has been shown as risk factor for metabolic syndrome in adolescents [215]. SSB intake was shown to be positively associated with waist circumference and proportion of visceral to subcutaneous fat with no change in total body fat [60], indicating a restructuring of body fat depots. Furthermore, in a six month intervention study, one liter of SSB per day resulted in higher liver fat, skeletal muscle fat, blood triglycerides, and VAT in adults [59]. A minority cohort study demonstrated that VAT accumulation is greatest during young adulthood in NHB and Hispanic populations [217], and therefore adolescence is an important time period to reduce VAT and SSB intake to minimize metabolic syndrome risk, especially in minorities.

The relationship between SSB and VAT appears to be more nuanced than a simple increase in caloric intake from SSB. Research has shown that intake of SSB is generally not compensated for at later meals[143]. Additionally, evidence indicates that the total intake of calories is greater than those from the added SSB consumption alone, possibly indicating a change in satiety [144]. Recent studies indicate that the stress hormone cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA) axis, may mediate the relationship between sugar intake and fat partitioning. Previous work has found dietary sugar intake and cortisol predicted VAT accumulation in NHB and Hispanic youth (13-18 years of age) [228]. In overweight adolescents, cortisol levels were associated

with higher regional fat mass and insulin resistance [45]. In a sample of only overweight Hispanic youth (8-13 years of age), youth with higher morning serum cortisol levels had higher prevalence of metabolic syndrome [46]. In the same adolescent Hispanic sample, morning serum cortisol was negatively associated with a 2-year decline in insulin sensitivity and beta cell function, both of which are pathophysiological underpinnings of metabolic disease,[7]. Therefore, the purpose of this study is to examine the interrelationships between of SSB consumption on VAT and CAR in Hispanic and NHB youth. Our *a priori* hypotheses are: high SSB intake will show a dose response relationship with increasing VAT, increasing SSB will be related to increased CAR, and that the interaction of SSB and CAR will yield differential outcomes on VAT.

SUBJECTS AND METHODS

Participants

Two hundred and forty-one overweight and obese adolescents (8-17 years of age) were recruited from hospitals, clinics, churches, schools, and community centers around the Los Angeles area between 2008 through 2011 into the initial study (Diabetes Risk due to Ectopic Adiposity in Minority Youth)[219]. All subjects were ethnically Hispanic (all four grandparents of Latin-American descent) or self-identified NHB, 8–17 years of age, and either overweight or obese (≥ BMI 85th percentile for age and sex) [301,302]. For this subset analysis (n=91) the following inclusion criteria were included: Tanner stage 4 or 5 (post-puberty)[303], 13-17 years of age, and having had at least two valid diet recalls within a month of the testing visit. Subjects were excluded if they had a chronic illness (e.g. type 1 or type 2 diabetes), were currently in a weight loss program, or used medications that

influenced insulin or body composition. Informed written parental consent and participant assent was obtained before any testing commenced. The Institutional Review Board of the University of Southern California (USC) approved all study procedures.

Procedures

Subjects attended two visits to the Clinical Trials Unit (CTU) at USC. At the initial visit, a licensed health care professional provided a physical exam for the subjects. Tanner stage was determined via breast stage for females and pubic hair stage for males [304,305]. The testing visit took place within two months of the initial visit. At the second visit, subjects were admitted and fed dinner and a snack before 8:00pm. After 8:00pm, the subjects began an overnight fast.

To examine salivary cortisol patterns, CTU staff collected three saliva samples at 5:30am, 5:45am, and 6:00am. Samples were collected using the Salivette system (Sarstedt, Newton, NC). Saliva samples were immediately centrifuged at 2500 rpm for 10 min and then frozen at-70 degrees C until assayed. The cortisol awakening response was calculated as the difference between the 5:30 and 6:00am time points [302]. CAR is a commonly used measure of morning variation and has been associated with disease outcomes[246,306–309].

Height and weight were measured to the nearest 0.1 kg and 1cm, using a beam medical scale and wall-mounted stadiometer. BMI was calculated as weight (kg) divided by height (m) squared. BMI percentiles were determined using the Center for Disease Control age-and-sex specific values, and were calculated with EpiInfo 2005 (Center for Disease Control, Atlanta, GA).

All subjects underwent abdominal MRI scanning to determine fat deposition on a commercial 3-Tesla MRI system (Excite HD, GE Health- care, Waukesha, WI, USA). Fat quantification for visceral adipose tissue (VAT) abdominal scans was previously described [219] and has been validated in numerous studies [310–313]. Stress was assessed using the Perceived Stress Scale (PSS), a 14-item survey that inquires about perception of stressful experiences in the preceding month [314].

Eighty-one subjects had VAT data. CAR was available for 60 of the 81 with VAT. Student T tests were performed to insure there were no differences between the CAR subset and the larger VAT dataset.

Dietary Assessment

Dietary intake was assessed over the phone via the multiple pass 24 hour recall method using Nutrition Data System for Research (NDS-R)(University of Minnesota, Minneapolis, MN), which has been validated here [315]. Recalls were performed within one month of the testing visit. To be included in the analysis, subjects needed at least two valid recalls, subjects with less than two recalls or recalls more than a month before the testing visit date were excluded. Beverages considered SSBs were: sweetened soft drinks, sweetened fruit drinks, sweetened tea, sweetened coffee, sweetened coffee substitutes, and sweetened water. A serving was defined as 8 fluid ounces. Subjects were classified into SSB categories based on dietary recall data: low SSB consumers (< 1 servings per day), medium SSB consumers (≥1 - <2 servings per day), and high SSB consumers (≥2 servings per day). These categories were chosen to model an increase in SSB intake by one serving. The American Heart Association (AHA) recommends consuming under 150

kcal/day from added sugar for men and under 100 kcal/day[316]. Since a single serving of SSB can contain anywhere from 100 to 180 kcal, the SSB groups additionally represent an adherence to the AHA's recommendations. SSB was additionally modeled as a categorical variable, rather than a continuous, to minimize reporter bias. Dietary data was also checked for unusual or implausible caloric values via linear regression of caloric intake versus BMI. All subjects had energy intake residuals less than 2 standard deviations from the mean, and therefore all subjects were included in this analysis.

Statistical Analysis

R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses. Perceived stress, VAT, energy intake, and BMI percentile were square root transformed to satisfy normality assumptions. Perceived stress, Tanner stage, ethnicity, sex, BMI percentile, and energy intake were included as covariates. Analysis of covariance (ANCOVA) was used to assess differences in CAR and VAT (dependent variables entered separately) between SSB groups (independent variable), and the following *a priori* covariates were entered: ethnicity, Tanner stage, BMI percentile, sex, and energy intake (calories per day). All covariates were chosen because of previous research indicting possible relationships to either VAT[228] or CAR[317,318] . SSB by VAT (SSB*VAT) or SSB by CAR (SSB*CAR) interactions were also added to their respective model. In all models, the low SSB group represented the reference category. Log-likelihood ratio tests were used to compare the relative fit of the model after the addition of the SSB*VAT or SSB*CAR interaction term. Tukey post hoc tests were used to evaluate between SSB group differences.

The adjusted means are reported in **Figure 4.1** and **Figure 4.2**. Statistical significance was set at $p \leq 0.05$. The beta value (β) refers to the estimated regression coefficient; d refers to the Cohen's d as a measure of effect size between factor means.

RESULTS

Table 4.1 summarizes the demographics and adiposity variables for subjects with two valid diet recalls by SSB group and outcome variable. Only CAR and daily energy intake differed by SSB category (both p=0.01) for both the CAR and VAT analysis.

Table 4.1: Demographics, adiposity, cortisol, and dietary intake variables of study sample

	Total	**Low SSB (<1serv/day)**	**Medium SSB (1≥serv<2/day)**	**High SSB (≥2serv/day)**
N	60	31	9	20
Sex (Female/Male)	28/32	14/17	4/5	10/10
Tanner (stage 4/stage 5)	14/46	10/21	2/7	2/18
Ethnicity (NHB/Hispanic)	18/42	11/20	1/8	6/14
BMI z-score	2.1±0.4	2.2±0.4	2.1±0.5	2.0±0.5
Visceral Adiposity (L)	1.9±1.0	1.8±1.0	1.9±1.1	2.1±1.1
Subcutaneous Adiposity (L)	8.4±3.5	8.5±3.3	8.3±4.5	8.1±3.5
CAR (μg/dl)	0.9±0.6	0.6±0.6	0.8±0.7	1.2±0.6
Energy Intake (kcal/day)	1710±608	1502±618	1753±506	2061±544

Data are presented as n or mean ± standard deviation

After adjusting for covariates, the high SSB intake group exhibited a 7% higher VAT compared to the low SSB intake group (β=0.25, CI:0.03, 0.33, p=0.02) (**Figure 4.1**), with a medium effect between high and low SSB groups (d=-0.55, p_{Tukey}=0.03).

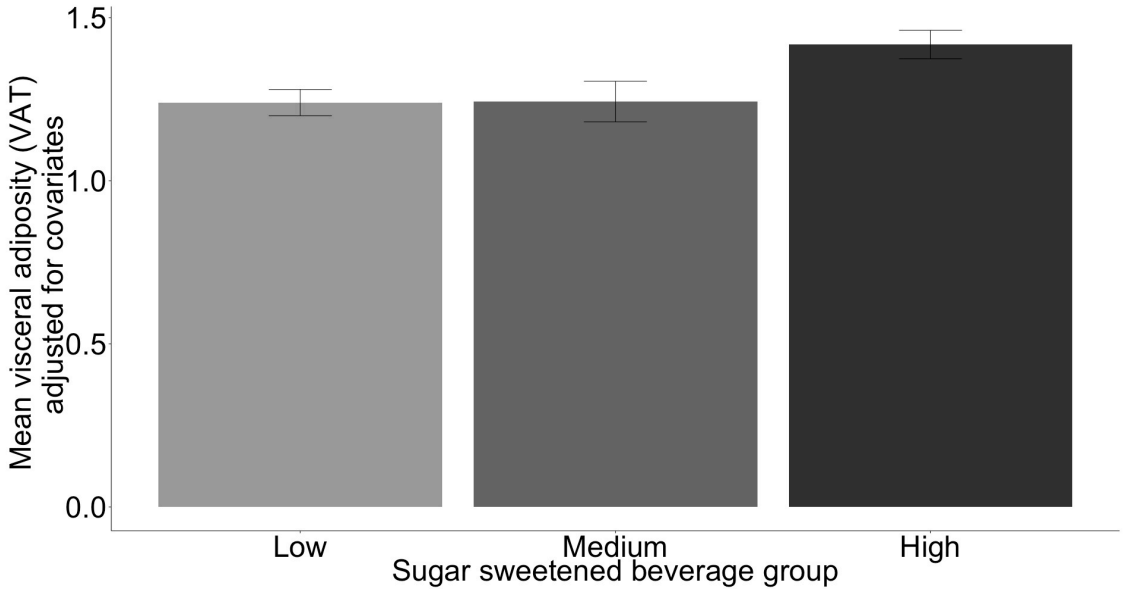

Figure 4.1: Mean cortisol awakening response adjusted for sex, BMI, tanner stage, ethnicity, and energy intake by sugar sweetened beverage consumption categories. Error bars represent plus and minus one standard error. * $p \leq 0.05$.

VAT was also associated with ethnicity (p=0.01), sex (p=0.01), and BMI (p=0.01). Post hoc tests revealed the high exhibited greater CAR compared to the low group (p=0.05). The addition of CAR improved the fit of the model (0.01). CAR was associated with VAT (β=0.31, CI=(0.01,0.23), p=0.02). The interaction term SSB*CAR did not significantly improve the model fit (**Table 4.2**).

Table 4.2. Analysis of covariance (ANCOVA) models for Cortisol Awakening Response (CAR) and Visceral Adipose Tissue (VAT).

	CAR			VAT		
	Beta	95% CI	P	Beta	95% CI	P
Model 1						
SSB Low	Reference category			Reference category		
SSB Medium	0.07	-0.48, 0.48	0.99	0.03	-0.17, 0.20	0.88
SSB High	0.30	0.02, 0.48	0.04	0.25	0.03, 0.33	0.02
Sex	-0.28	-0.70, -0.01	0.04	0.29	0.09, 0.36	<0.01
BMI	-0.08	-0.47, 0.22	0.48	0.53	0.30, 0.58	<0.01
Tanner	-0.05	-0.41, 0.42	0.96	0.03	-0.14, 0.18	0.76
Ethnicity	0.02	-0.29, 0.41	0.74	0.47	0.27, 0.55	<0.01
Energy Intake (kcal/day)	0.23	-0.14, 0.71	0.19	-0.22	-0.31, 0.02	0.08
Perceived stress	0.27	-0.11, 0.51	0.20	-0.07	-0.15, 0.09	0.65
Tukey adjusted means						
SSB High-Low		-0.50, 0.61	0.09		-0.01, 0.36	0.05
SSB High-Medium		-0.19, 0.94	0.23		-0.06, 0.40	0.21
SSB Medium-Low		-0.50, 0.61	1.00		-0.21, 0.24	0.99
Model 2	Beta	95% CI	P	Beta	95% CI	P
SSB Low	Reference category					
SSB Medium	-0.01	-0.47, 0.45	0.96	0.03	-0.16, 0.19	0.88
SSB High	0.21	-0.13, 0.66	0.18	0.19	-0.02, 0.28	0.09
Sex	-0.37	-0.91, -0.16	<0.01	0.35	0.13, 0.41	<0.01
BMI	-0.33	-0.93, -0.02	0.03	0.59	0.32, 0.59	<0.01
Tanner	-0.01	-0.41, 0.39	0.95	0.02	-0.13, 0.18	0.76
Ethnicity	-0.17	-0.71, 0.16	0.22	0.48	0.27, 0.53	<0.01
Energy Intake (kcal/day)	0.21	-0.02, 0.82	0.06	-0.34	-0.34, -0.02	0.02
Perceived stress	0.17	-0.07, 0.52	0.14	-0.07	-0.17, 0.06	0.37
VAT	1.10	0.12, 1.48	0.02			
CAR				0.31	0.01, 0.23	0.02
Interaction terms						
SSB*VAT	-0.30	-1.39, 0.54	0.66			
SSB*CAR				0.09	-0.11, 0.24	0.12

Significant value p≤0.05; [a] Sample size=60

The high SSB intake group exhibited 22% higher CAR compared to the low SSB intake group (β=0.30, CI:(0.02,0.48), p=0.04) (**Figure 4.2**).

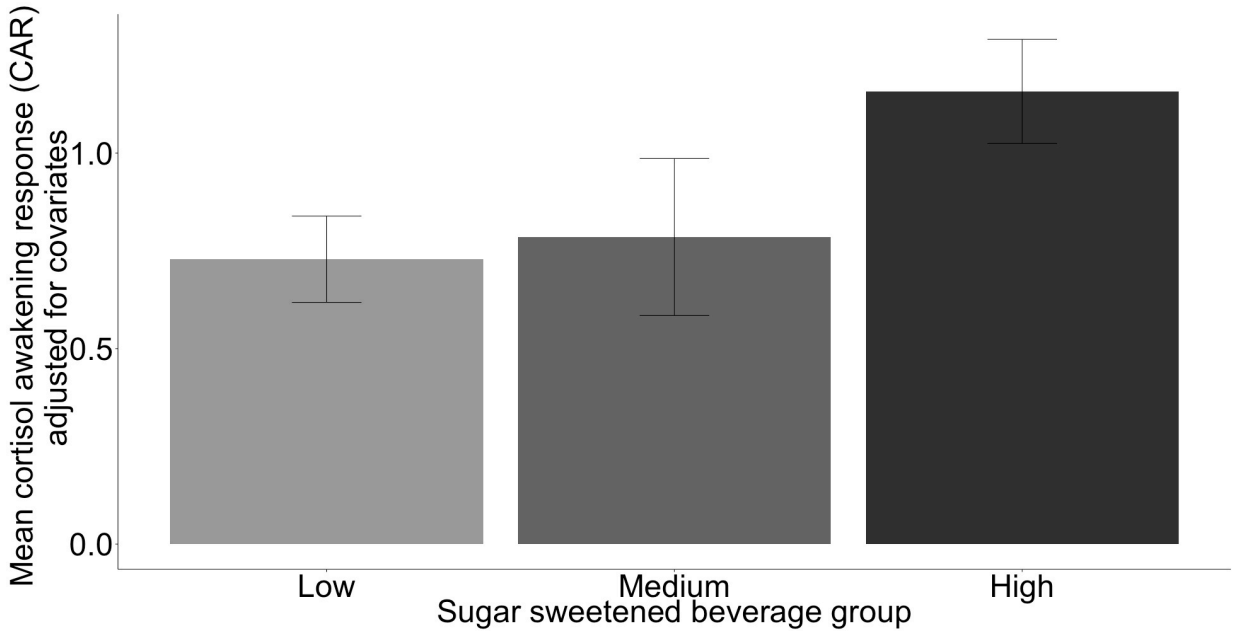

Figure 4.2: Mean visceral adipose tissue adjusted for sex, BMI, tanner stage, ethnicity, and energy intake by sugar sweetened beverage consumption categories. Error bars represent plus and minus one standard error. * $p \leq 0.05$.

There was a smaller (but significant) effect between the high and low SSB groups (d=-0.64, p=0.03). CAR was also associated with sex (p=0.04). VAT improved the fit of the model (p=0.01). VAT was associated with CAR (β=1.10, CI:(0.12,1.48), p=0.02). The interaction term SSB *VAT did not significantly improve the model (**Table 4. 2**).

DISCUSSION

The contrary to the hypothesis, there was no interactive effect between VAT, CAR, and SSB, rather independently consuming over two servings of SSB per day intake was related to higher VAT and CAR in overweight and obese minority youth compared to drinking less than one serving of SSB per day. The effect of CAR was the strongest predictor of VAT (and vice versa), however SSB appears to influence both and therefore is an attractive intervention target to reduce both CAR and VAT.

This is the first paper examining the main effect of SSB on CAR or any biological marker of HPA axis activity. Much of the previous work between CAR and sugar intake, or sweet perception, has focused on the mediating effect of stress[52,246,319–321]. However this study saw no effect of perceived stress on SSB intake nor CAR. The association between elevated CAR and SSB consumption could be a result of chronic elevated cortisol due to metabolic dysregulation increasing the desire to consume SSB. Treatment with exogenous glucocorticoids has been shown to increase food intake [52], and chronic glucocorticoid exposure has been related to increased palatable food intake [322,323], in particular from sweet foods compared to savory foods [324]. In pathological glucocorticoid exposure, seen in Cushing's disease, patients exhibited increased sweet craving and decreased post meal satiation [325]. Furthermore, patients with Cushing's Disease, VAT was associated with increased post meal hunger [325], also indicating a relationship between dietary consumption cortisol and VAT. The current findings may be modeling a sweet preference due to elevated cortisol.

Additionally, this study showed an increase in VAT with SSB intake. Previous research has implicated SSB consumption, independent of energy intake, in promoting VAT accumulation [59–61,75,116], although, a majority of this research has taken place in adults [59–61,75]. However, adolescence in particular appears to be a critical period in VAT accumulation associated with SSB intake [116], with VAT accrual peaking in young adulthood in minority populations [217]. Zheng and colleagues showed in a longitudinal study that SSB consumption at 15 years of age was associated with higher increases in BMI and waist circumference after 6 years, moreover this relationship was only seen at

age 15 and not in younger (pre adolescent) age groups [116]. The current study supports these findings, showing consumption of more than 2 SSB per day was associated with increased VAT after adjusting for daily energy intake and BMI. The mechanism behind the association of SSB intake and VAT has yet to clarified, however sweetener-type may be related. Stanhope and colleagues have shown fructose beverages, compared to glucose beverages balanced for caloric load, to be positively related to increased VAT [75]. Specifically suggesting that because fructose is directly metabolized in the liver, it increases triglyceride production in the liver which further leads to preferential increase in VAT [75]. A large study of 559 adolescents ages (14-18 years) found that fructose intake was related to VAT, and that VAT mediated fructose related associations with cardiometabolic risk factors [137]. Since popular SSBs have been shown to contain upwards of 60% fructose [72], it is possible that in addition to modeling an increase in SSB intake due to sweet preference, this study is also modeling a snapshot of fat repartitioning due to high fructose intake.

Furthermore, this study showed a moderate positive relationship between CAR and VAT. The VAT itself may provide a mechanism to the elevated CAR. Glucocorticoid receptors in VAT have been linked to heightened local cortisol production and receptor expression [43]. Likewise, high glucocorticoid levels have been linked to increased de novo lipogenesis in coordination with high insulin levels, and this in turn influences fat partitioning [32643]. Interestingly, this increase in de novo lipogenesis is similar to the fructose mechanism speculated above [75]. The combination of high CAR, VAT, and high fructose intake from SSB may create a metabolic storm, particularly in

the liver. Work in similar samples supports this metabolic storm hypothesis, Gyllenhammer and colleagues found that total and added sugar intake increased the relationship between cortisol and VAT in a similar sample of NHB and Hispanic adolescents [228]. Furthermore, cortisol has been shown to be negatively associated with insulin sensitivity in Hispanic children (8-13 years), and total sugar intake were further shown to be related to increased fat mass and lower insulin sensitivity in Hispanic youth (10-17 years) [327]. Finally, in Hispanic youth ages (8-13 years) metabolic syndrome was associated with increased morning cortisol [46]. While the above studies and this study are all cross-sectional, there appears to be a relationship between VAT, CAR, and dietary sugar intake, in particular SSB, on metabolic health in minority youth.

There are several limitations to note. As previously mentioned this is a cross-sectional study and causality cannot be inferred. This study also has a small sample size, especially for the cortisol variables. Additionally, this study did not take into account sleep variables, which could further elucidate the relationship between VAT, CAR, and SSB. Having only two recalls limits dietary data quality, however use of a categorical SSB variable is meant to minimize reporter bias. This study should be repeated with an increased sample size and three or more dietary recalls. Additionally, nocturnal, urinary, and serum cortisol were available, however were not used due to small sample sizes. Finally, the CAR reading is based on only one day and may not be representative of the average CAR of the individual.

SSB consumption appears to be independently associated with higher VAT and higher CAR in overweight and obese minority youth. This study highlights high fructose

corn syrup consumption as a potential moderator of VAT and CAR. Additionally, SSB is an attractive and highly modifiable target for interventions looking to decrease CAR and VAT in a minority youth population. Additionally, further work is needed to elucidate the possibility of CAR increasing desire for palatable foods, as well as the possibility of a positive feed back loop between SSB consumption, VAT accumulation, and CAR elevation.

Chapter 5: The association between added sugar intake and perceived hunger in Hispanic children

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(In preparation for Appetite)

GES was responsible for data collection, data analysis, and manuscript preparation.

ABSTRACT

Background: Perceived hunger is a noted barrier to weight loss in children. Hispanic children in particular are at risk for obesity as well as overeating. This overall goal of this study was to examine the relationship between metabolic parameters (insulin, cortisol, adipokines) on hunger and satiety at an ad libitum meal. Additionally, this study assessed the relationship between dietary intake both free-living and at an ad libitum meal on hunger and satiety in Hispanic children (7-10 y).

Methods: This was a cross-sectional experiment with 41 overweight or obese Hispanic children (7-10 y). The experiment included three 24-hour recalls to assess free-living dietary intake. At a testing visit, the children were offered an ad libitum breakfast after an fMRI scan. Throughout the testing visit blood was drawn six times and hunger and satiety were assessed via visual analogue scale at the same six time points as well. Free-living macronutrient intake and at ad libitum meal, free-living dietary variables, plasma insulin, plasma glucose, plasma cortisol, baseline adipokines, and body composition were assessed for their relationship to satiety or hunger. For this analysis, the subjects were grouped into the following tertiles added sugar intake at the ad libitum meal groups: subjects consuming under 5% of daily calories from added sugar as low intake group (n_{ad}

$_{libitum}$=14), subjects consuming 5% to 10% of calories from added sugar as a medium consumers ($n_{ad\ libitum}$=14), and subjects consuming more than 10% of calories from added sugar as a high consumer ($n_{ad\ libitum}$=13). The subjects were grouped into the following tertiles of added sugar intake free-living groups: subjects consuming under 8% of daily calories from added sugar as low intake group ($n_{free\ living}$=11), subjects consuming 8% to 11% of calories from added sugar as a medium consumers ($n_{free\ living}$=15), and subjects consuming more than 11% of calories from added sugar as a high consumer ($n_{free\ living}$=15). The subjects were additionally grouped into the following tertiles of saturated fat intake at the ad libitum meal groups: the low intake group consumed under 8% of their calories from saturated fat ($n_{ad\ libitum}$=14), the medium intake group consumed between 8% and 13% of there calories from saturated fat ($n_{ad\ libitum}$=14), and the high intake group consumed over 13% of their daily calories from saturated fat ($n_{ad\ libitum}$=13). The subjects were also grouped into the following tertiles of saturated fat intake free-living groups: the low intake group consumed under 10% of their calories from saturated fat ($n_{free\ living}$=12), the medium intake group consumed between 10% and 13% of there calories from saturated fat ($n_{free\ living}$=15), and the high intake group consumed over 13% of their daily calories from saturated fat ($n_{free\ living}$=14). Finally, the subjects were grouped into the following tertiles of fiber free-living intake groups: the low group consuming less than 11 grams ($n_{free\ living}$=13), medium group consuming between 11 and 15 grams ($n_{free\ living}$=14), and the high fiber intake group consuming 15 or more grams of fiber per day ($n_{free\ living}$=14). Mixed modeling was used to explore how dietary intake and metabolic parameters predicted hunger/satiety ratings across time. The following a priori covariates

were included: sex, age, body fat percentage, energy intake (calories/day) at the ad libitum meal, and waist circumference.

Results: The low added sugar intake at the ad libitum meal group was less hungry compared to the high-added sugar intake at the ad libitum meal group during the testing visit (β =26.2, CI: (6.6, 45.8), p=0.01). The low added sugar intake at the ad libitum meal group was on average more satiated than the high added sugar intake at the ad libitum meal group during the testing visit (β =-24.8, CI: (7.1, 42.6), p=0.01). No other free-living or ad libitum meal variables were related to hunger or satiety. None of the metabolic parameters were related to hunger or satiety. Waist circumference was related to satiety (β =-0.48, CI: (-0.8, -0.1), p=0.01).

Discussion: In young children, added sugar at the ad libitum meal appears to drive feelings of satiety and hunger. This could be due to sight of a highly preferred food increasing feelings of hunger.

INTRODUCTION

Hispanic minorities are disproportionately at risk for obesity, with 46% of Hispanic youth (6-11 y) being overweight or obese (BMI $\geq 85^{th}$ percentile) compared to only 29% of NHW peers [5]. Perceived hunger may be an important determinant in the development and maintenance of obesity. In adults [17] and children [22,23] perceived hunger has been a barrier to weight loss, as well as a possible risk factor for developing obesity [18]. In obese adults, perceived hunger predicted weight loss at 6 and 12 months during an intervention, with those who were less hungry having lower BMIs [328]. Children (5-6 y)

with poor appetite regulation were found to be more susceptible to obesegenic environments [329]. Furthermore, obese children and adolescents reported greater hunger compared to normal weight peers [330]. A study looking at overweight Hispanic children found they consumed more energy in the absence of hunger compared to non-overweight children, and this eating in the absence of hunger was highly heritable [331]. This suggests that dysregulation of hunger signaling may be particularly important for Hispanic children, especially considering their higher prevalence of obesity and increased risk of obesity related diseases, such as T2D and insulin resistance [7–12].

Discovery of dietary components related to hunger is an appealing method to tailor obesity preventative and treatment efforts. Much of the previous research that has examined the effects of either low or high glycemic meals on satiety measures in children has mixed results [287–291,332–334]. A study with children (9-12 y) found those who had a low glycemic breakfast ate less food at lunch compared to children who ate a high glycemic breakfast [332]. A more recent study of children (9-12 y) found no difference in satiety after a low glycemic index breakfast compared to a high glycemic index breakfast [333]. Additionally, in obese Hispanic children (7-15 y), a low glycemic meal compared to a high glycemic meal did not impact feelings of hunger or satiety nor did they consume less food at an ad libitum meal [334]. A study that examined more specific components of carbohydrate intake found that a sucrose preload compared to a fructose or glucose preload, resulted in an increase in subjective appetite, and the glucose preload suppressed food intake at a test meal in normal weight boys (9-14 y) [139]. In overweight and obese adolescents (13-17 y), sugar sweetened beverage intake of greater than two servings per

day was associated with decreased feelings of fullness compared to those who drank less than one serving per day [335]. Research has also examined the effect of protein intake on hunger and satiety, with results indicting protein has a modest satiating effect compared to carbohydrates in children [336–338]. In a randomized control crossover feeding study with 34 obese boys (10-12 y), addition of low fat milk at a breakfast increased satiety compared to a water or apple juice addition [339]. Along with protein, fiber has been consistently used to increase satiety [340–343]. Nearly all research in satiety has focused on acute diet manipulation on short-term satiety, often between a test breakfast and lunch, with few studies looking at free-living intake and its effect on satiety at a test meal.

Insulin action and adipokine response in Hispanic children has been shown to be altered in the overweight and obese state [165,203,207]. Increased leptin has been associated with increased satiety in normal weight adults [344]. Additionally, cortisol response has been shown to be altered by macronutrient intake in Hispanic youth [228] and has recently been implicated in non-homeostatic eating [50]. These circulating factors may be a missing link between dietary factors and appetite. A randomized control trial with 100 hyperinsulinaemic children (6-12 y) found that metformin reduced body fat and perceived hunger, indicating a possible relationship between perceived hunger and insulin status [345]. Additionally, a study, which manipulated the resistant starch composition of bread, found improvement in glycemic and insulinemic response as well as improved appetite ratings, further indicating a possible relationship between hunger, dietary intake, and metabolic parameters [346]. This overall goal of this study was to examine the relationship between metabolic parameters (insulin, cortisol, adipokines) on

hunger and satiety at an ad libitum meal. Additionally, this study assessed the relationship between added sugar intake both free-living and at an ad libitum meal on hunger and satiety in Hispanic children (7-10 y). Also, we assessed overall dietary intake, both free-living and at the ad libitum meal, on hunger and satiety. We hypothesized that high circulating insulin, cortisol, and leptin would be associated with increased hunger and that adiponectin would be associated with increased satiety. Furthermore, we hypothesized that high free-living sugar consumers compared to low free-living sugar consumers would have increased hunger and decreased satiety during the ad libitum meal independent of caloric intake or dietary intake during the meal.

SUBJECTS AND METHODS

Participants

Participants were recruited from local health clinics and by word of mouth around the Austin area. All participants were Hispanic (7-10 y) (four grandparents of Hispanic descent), and at or above the 85th percentile for BMI. Participants were excluded if they had magnetic resonance (MR) contraindications, were taking medications known to influence body composition, had type 1 or 2 diabetes, and or had any major illness known to influence body composition. The participants were also screened for disordered eating with the "Questions about making your self Sick, loss of Control, loss of One stone in three months, do you believe you are Fat, and would you say Food dominates your life" (SCOFF) eating disorder screening tool adapted for children in the United States [347]. The Institutional Review Board of the University of Texas at Austin approved this study.

Written informed consent was obtained for all participants from their legal guardians, as well as written assent from the participant before any testing was done.

Body Composition

Height and weight were measured to the nearest cm and kg on a seca-769 physician scale and stadiometer (Seca, Chico, CA). Body fat mass was assessed via Air Displacement Plethysmography (ADP) using a BodPod (Cosmed, USA Inc, Chicago USA).

Dietary recalls

Three multi-pass 24-hour recalls using NDS-R software (2014; Nutrition Data System for Research, University of Minnesota, Minneapolis, MN) were collected via telephone to assess free-living dietary intake. All three dietary recalls were performed within one week of the in-person testing visit.

Testing Visit

All testing visits started between 7 and 9 am. Participants were instructed to come to the testing visit after a 12 hour fast, nothing to eat or drink except water. Participants were called and texted the evening before the visit to remind them when to start fasting. At the start of the testing visit the participant was asked to indicate on a visual analogue scale (VAS) how hungry he or she felt and how great his or her desire to eat. Additionally at the start of the testing visit, a registered nurse placed an intravenous catheter (IV) in either the participant's ancubeital or cephalic vein. The nurse was

allowed three opportunities to place the IV. After three attempts, the participant was asked if he or she or she would like to continue. Five milliliters (ml) of blood was drawn initially into an EDTA coated vial. After the first VAS and blood draw the subject underwent a magnetic resonance imaging (MRI) session. After the MRI session, the subject was escorted to a private room for an ad libitum meal. The subjects were instructed to eat as much or as little as he or she desired for 40 minutes. All foods offered were weighed before the meal and after the meal to the nearest 0.1g. The VAS scale was repeated every ten minutes during the meal challenge. Five ml of blood was draw an additional five times (every 10 minutes) once immediately after the MRI and during the meal challenge. All blood was promptly refrigerated. At the end of the testing visit the blood was centrifuged and the serum was extracted and frozen at -80 degrees Celsius until it was analyzed. The participants were instructed to eat as much or as little as he or she desired. During the meal challenge the final four blood draws and VAS scales were completed every 10 minutes. The baseline fasted blood draws were assayed for leptin, adiponectin, resistin, glucose, cortisol, and insulin concentrations. The subsequent blood draws after the MRI were assayed for glucose, insulin, and cortisol only. Baseline plasma was assayed with a luminex using the human steroid hormone and human adipokine panels 1 and 2 (Millipore, Billerica, USA). Insulin was assayed using TOSOH 600 II analyzer (Origio,Målov, Denmark) and glucose was assayed using Sirrus analyzer (Stanbio, Boerne, TX, USA) using glucose oxidase reagent.

Statistical Analysis

All analyses were performed with R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Because the hunger, fullness, cortisol and insulin data was collected over time, linear mixed effects models were fit with a random intercept and random slope to assess the effect of added sugar and time on the two appetite variables (hunger, fullness) and insulin. In the model, the fixed effect of time was modeled using a spline with three degrees of freedom. The baseline models included the dependent variable of interest (hunger, fullness) and only the spline for time as the independent variable. Dietary variables listed in **Table 5.1** were independently modeled to test their relationship with hunger and satiety. At the ad libitum meal, subjects were exposed to a limited variety of foods, therefore food type, such as vegetables, fruit, and meat, were only modeled from free-living, where there were greater potential differences in diet.

Table 5.1: Dietary intake in free-living conditions and at the ad libitum test meal

Free living	Mean ±SD	IQR
Energy (kcal/day)	1517.6 ±431.1	708.8
Total carbohydrate (grams/day)	195.9± 67.8	104.7
Total protein (grams/day)	62.2± 21.9	27.5
Total fat (grams/day)	56.0± 19.1	29.4
Saturated fat (grams/day)	19.4± 8.0	10.3
Total sugar (grams/day)	81.7± 33.1	40.4
Added sugar (grams/day)	38.9± 23.4	24.9
Total fiber (grams/day)	31.8± 6.2	6.7
Vegetable intake* (servings/day)	1.1± 0.8	1.2
Fruit intake (servings/day)	1.7± 1.9	1.2
Sugar sweetened beverage intake (servings/day)	0.5 ± 0.7	0.6
Meat intake (servings/day)	2.1± 1.4	1.5
Dessert intake (servings/day)	0.6± 0.5	0.7
Dairy intake (servings/day)	1.5± 1.1	1.4
Refined grain intake (servings/day)	3.8± 2.1	2.4
Whole grain intake (servings/day)	1.2± 1.3	1.6
Fried vegetable intake (servings/day)	0.2± 0.5	0.1
Ad libitum meal		
Energy (kcal/day)	631.4± 411.8	524
Total carbohydrate (grams/day)	92.4± 57.4	51.0
Total protein (grams/day)	12.0± 10.8	9.9
Total fat (grams/day)	25.8± 20.2	28.5
Saturated fat (grams/day)	8.3± 7.2	9.6
Total Sugar (grams/day)	51.7± 35.1	32.2
Added Sugar (grams/day)	34.2± 26.4	28.8
Total fiber (grams/day)	6.1± 5.4	3.3

*Does not include potatoes
SD=Standard Deviation; IQR=interquartile range

Added sugar consumption was divided into tertiles of intake for both the free-living intake and at the ad libitum meal. At the ad libitum meal the categories were as follows: subjects consuming fewer than 5% of daily calories from added sugar as low intake group ($n_{ad\ libitum}$=14), subjects consuming 5% to 10% of calories from added sugar as medium consumers ($n_{ad\ libitum}$=14), and subjects consuming more than 10% of calories from added sugar as a high consumer ($n_{ad\ libitum}$=13). The World Health Organization (WHO) recommends no more than 10% of daily calories from added sugar [316], and the high group represents children who exceed that recommendation.

The free-living added sugar categories were as follows. Subjects consuming fewer than 8% of daily calories from added sugar as low intake group ($n_{free\ living}$=11), subjects consuming 8% to 11% of calories from added sugar as medium consumers ($n_{free\ living}$=15), and subjects consuming more than 11% of calories from added sugar as high consumers ($n_{free\ living}$=15). The World Health Organization (WHO) recommends no more than 10% of daily calories from added sugar [316], and the high group represents children who exceed that recommendation.

Saturated fat intake was also divided into tertiles of intake for both for free-living intake and at the ad libitum meal. At the ad libitum meal the categories were as follows. The low intake group consumed under 8% of their calories from saturated fat ($n_{ad\ libitum}$=14), the medium intake group consumed between 8% and 13% of there calories from saturated fat ($n_{ad\ libitum}$=14), and the high intake group consumed over 13% of their daily calories from saturated fat ($n_{ad\ libitum}$=13). The World Health Organization

recommends limiting saturated fat intake to under 10% of daily calories, and the low group represent those children who meet this recommendation [348].

Free-living saturated fat intake was as follows. The low intake group consumed under 10% of their calories from saturated fat ($n_{free\ living}$=12), the medium intake group consumed between 10% and 13% of there calories from saturated fat ($n_{free\ living}$=15), and the high intake group consumed over 13% of their daily calories from saturated fat ($n_{free\ living}$=14). The World Health Organization recommends limiting saturated fat intake to under 10% of daily calories, and the low group represent those children who meet this recommendation [348].

Additionally, total fiber intake was split into tertiles, with the low group consuming less than 11 grams ($n_{free\ living}$=13), medium group consuming between 11 and 15 grams ($n_{free\ living}$=14), and the high fiber intake group consuming 15 or more grams of fiber per day ($n_{free\ living}$=14). Fiber intake in this population was low, with all categories bellow the daily recommended intake for fiber intake which is between 25-31 grams for children [349]. Because total fiber at the ad libitum meal was very low (mean 6.1±5.4), ad libitum meal fiber intake was not split into tertiles.

The following additional variables were added as covariates in a stepwise fashion: BMI percentile, sex, and energy intake at the ad libitum meal (calories), percent body fat, waist circumference, fasting leptin, fasting resistin, and fasting adiponectin. Multiplicative interaction terms were added to the models according to individual independent variable significance also in a stepwise fashion. Continuous independent variables and covariates were square root or log transformed to satisfy normality

assumptions as needed. Log-likelihood tests were used to compare the relative fit of nested models after each stepwise categorical variable addition. Only variables, which improved the predictive ability of the model, were included. Bonferroni corrections were used to correct for multiple comparisons. Statistical significance was set at $p \leq 0.05$ for all models and beta value (β) refers to the regression coefficient.

RESULTS

Participant Characteristics

Forty-one participants completed the study, 22 female and 19 male participants. Overall, the sample was generally obese, 65% of the sample had a BMI≥ 95%. **Table 5.2** summarizes the demographics of the sample.

Table 5.2: Demographics

Demographic	
Body mass index percentile	96.5±2.5
Overweight/Obese	14/27
Age	8.8±1.1
Sex(F/M)	22/19
Percent body fat[a]	33.5±6.5
Waist circumference (cm)[a]	78.1±11.4
Leptin (pg/mL)	17223.8±9859.5
Resistin (pg/mL)	189.8±70.1
Adiponectin (pg/mL)	67918.1±51293.8
Insulin (uU/mL)	46.2±56.9
Cortisol (ng/mL)	149.3±65.2

N=41; [a]N=32

Added Sugar Intake

Although free-living added sugar intake was not associated with hunger or satiety, added sugar categories at the ad libitum meal significantly explained variability of hunger ($p<0.01$). The low added sugar intake group was less hungry compared to the high-added sugar intake group during the testing visit (β =26.2, CI: (6.6, 45.8), p=0.03). This difference in added sugar consumption is visualized in **Figure 5.1** as the additive shift of the added sugar group curves.

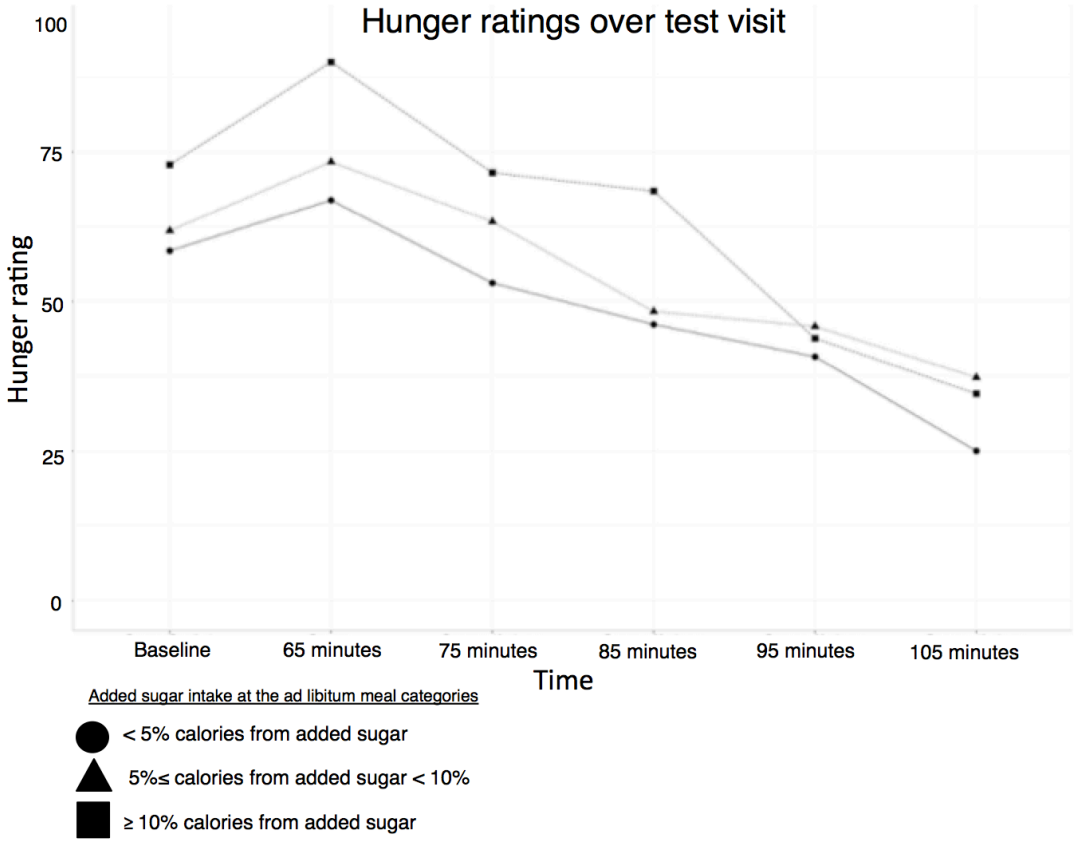

Figure 5.1: Hunger rating over the testing visit. High added sugar consumer at the ad libitum meal exhibited higher hunger over all than the low added sugar consumer at the ad libitum meal group (p=0.03). The difference is a visualized as a shift of the high added sugar curve compared to the low added sugar curve.

Additionally, added sugar categories at the ad libitum meal were the only dietary variable to significantly explained variability of satiety ($p<0.01$). The low added sugar intake group was on average more satiated than the high added sugar group during the testing visit (β =-24.8, CI: (7.1, 42.6), p=0.03). This difference in added sugar consumption is visualized in **Figure 5.2** as the additive shift of the added sugar group curves.

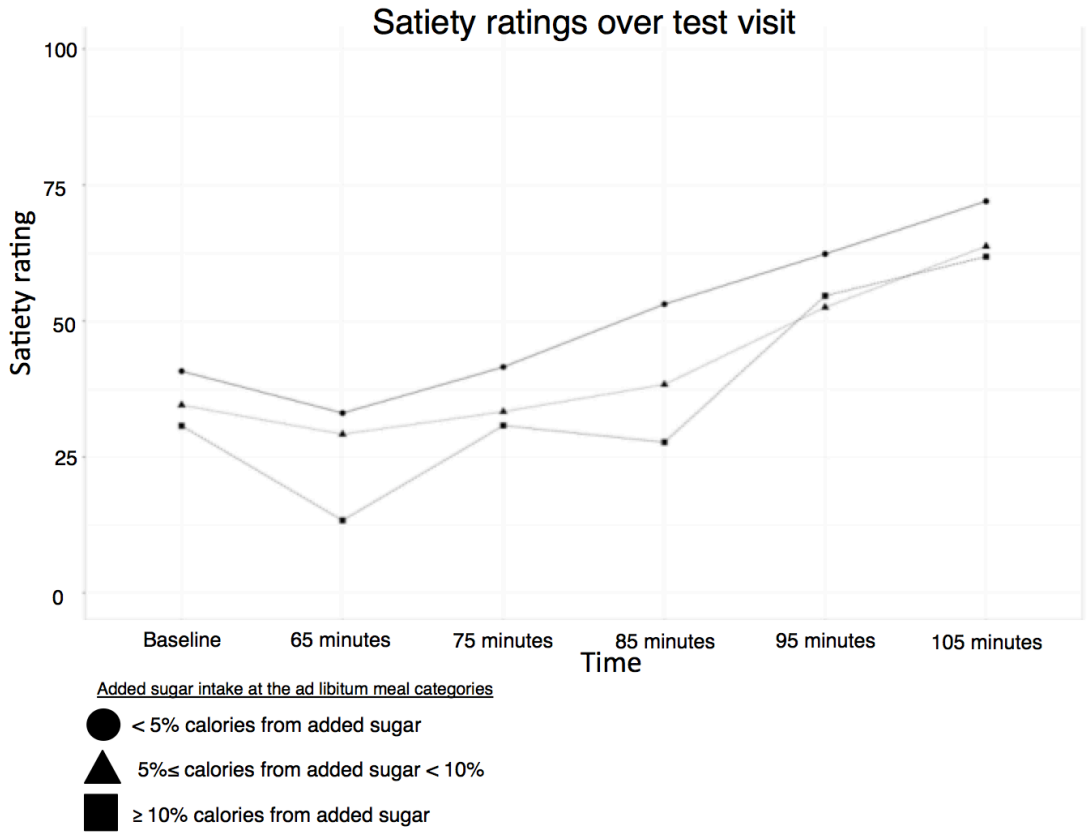

Figure 5.2: Satiety rating over the testing visit. High added sugar consumer group at the ad libitum meal exhibited higher hunger overall than the low added sugar consumer at the ad libitum meal group (p=0.03). The difference is a visualized as a shift of the high added sugar curve compared to the low added sugar curve.

Metabolic Parameters

No metabolic parameters (insulin, cortisol, glucose, leptin, resistin, or adiponectin) were related to hunger at the ad libitum meal. Of the other covariates (sex, BMI percentile, energy intake at the ad libitum meal, percent body fat, and waist circumference) only waist circumference was related to satiety (β =-0.48, CI: (-0.8, -0.1), p=0.01).

Dietary Parameters

Secondary analysis of free-living dietary variables showed no free-living dietary variables were related to hunger or satiety.

DISCUSSION

Added sugar intake at the ad libitum meal was the only dietary variable (free-living or at the ad libitum meal) associated with increased hunger and greater desire to eat in children independent of energy intake or homeostatic factors. In this analysis, no homeostatic factors were related to hunger or satiety. Of the adiposity measures, waist circumference was negatively associated with hunger. These findings suggest that perceived hunger and satiety are related to availability and desire to eat palatable food in children (7-10y).

Previous research has shown that children with greater appetites and decreased ability to control satiation consume bigger portions [329], which has in turn been linked to increases in BMI [350]. Additionally, many studies in children have examined overweight and obese children compared to lean children, showing obese children (5-12 y [351 352]) to exhibit lower internal satiety cues compared to lean. This study builds on those studying appetite within an overweight and obese population, and shows that appetite varies within overweight and obese children and that dietary intake impacts satiety/hunger levels. In Hispanic children (5-18 y), eating in the absence of hunger was shown to be highly heritable and that overweight children consumed more calories from eating in the absence of hunger [331]. This current study postulates that Hispanic children (7-10 y) who consume high amounts of added sugar feel hungrier in an ad libitum meal setting. Therefore, they

may be at risk of two phases of over eating, both in the absence of hunger and perception that they are hungrier.

What hunger is and how it is recognized is poorly understood. Generally, hunger is recognized as a motivating factor for consuming food. For half a century the Mayer's glucostatic theory has been the preeminent hypothesis for food intake [19]. The glucostatic theory posits that as blood glucose decreases, hunger increases, when blood glucose is restored to normal values, hunger decreases, and this process is regulated through the hypothalamus [19]. However, in the general population hypoglycemia is signaled by shaking, sweating, and weakness often without the person feeling "hungry" [20]. Conversely perceived hunger is more closely associated with gastric distension, circadian rhythms (feeling hungry because it is the normal meal time), and food reward (feeling hungry because something looks desirable) [20]. This research supports the latter hypothesis, that in children availability of a high sugar food motivates perceived hunger and desire to eat independent of hormonal signaling. Possible mechanisms for hunger independent of hormonal signaling is that children have uncoupled hunger from metabolic signals and rather rely on external signaling for appetite feelings. In normal weight adults, high sugar and high fat foods were rated as more satiating than low sugar and low fat snacks with similar energy content [353]. Additionally, sight of a highly preferred food increased feelings of hunger that remained high during consumption of the preferred food compared to less preferred food [354]. In children (11-12 y), the satiating qualities of foods appear to be related to the child's familiarity with the food [355]. Therefore, the children who felt hungrier may also have been familiar with foods with

high added sugar content, this could in part be related to the increased advertisement of high sugar foods targeted at children [356]. Future analysis should examine screen time as a possible covariate for hunger related to added sugar intake at the ad libitum meal. While the cross-sectional nature of the data prevents inferring the directionality of the relationship, added sugar intake and hunger appear to be related and this could be due to the child choosing a familiar and preferred food when hungry and the desire to eat the preferred food prevents satiating feelings.

Another notable finding from this analysis is the difference between free-living added sugar intake and ad libitum meal intake of added sugars. The subjects at the ad libitum meal consumed 87% of their free-living added sugar intake at a single meal. This further suggests a direction to the added sugar intake and hunger relationship. The availability of the added sugar at the ad libitum meal increased sugar consumption to a level nearly that of daily free-living intake, and this availability may have increased the desire to eat more sugar. These findings suggest that children may not know the difference between desire to eat and physical hunger. This aligns with previous research indicating that children who have low satiety responsiveness consume higher portions at eating occasions [329,357]. An additional possible mechanism could be the lack of parental oversight during the ad libitum meal. The children may rely on parental influence for portion control, as parenting style has been shown to influence child eating behavior [358–361]. More research is needed to elucidate the influence of parenting on added sugar intake in the absence of the parent. However, overall the subjects in this study consumed high

amounts of added sugar compared to daily free-living added sugar consumption and this could be driven by a desire to eat sugary foods interpreted as hunger.

This study also found that waist circumference was inversely related to satiety. This is in agreement with previous by Fearnbach and colleagues, which has shown intake at an ad libitum meal was related to regional fat distribution [362]. However, unlike Fearback's study, this analysis found a preference for sweet foods not savory foods. A possible reason for the difference could be the meal timing, as Fearback's test meal was a dinner, and this was a breakfast test meal. Children may prefer savory foods at dinner, but prefer sweet foods at morning meals. Additionally, it is interesting to note that the relationship between waist circumference was independent of weight status and body fat percentage, suggesting abdominal fat may effect perceived satiety.

There are several limitations to note in this study. Because this is a cross sectional study it is not possible to determine if the access to added sugar foods increased hunger, or if children with increased hunger ate more added sugar. Additionally, this study has a small sample size, which may have resulted in low power to detect other findings. Furthermore, this sample contained only overweight and obese Hispanic children, and may not be generalizable to non-Hispanic populations. Future studies should be conducted with a larger sample size to test the effect of added sugar at an ad libitum meal on perceived hunger.

To conclude, added sugar intake is associated with increased hunger and decreased satiety in overweight and obese Hispanic children. The mechanism behind why certain children consumed higher amounts of sugar and experienced increased hunger

and decreased satiety may be an important facet in the treatment and prevention of obesity. The high amount of sugar consumed could be due to familiarity with high sugar foods or a belief that those foods with higher sugar would be more satiating. Additionally, the increase in desire to consume sugary foods may increase hunger and decrease satiety, putting these children at an increased risk for weight gain. Finally, eating in the absences of parental control may promote sugar intake. Given that Hispanic children are at increased risk for disinhibited eating, interventions should focus on decreasing added sugar intake as a two-fold method of decreasing energy consumption and possibly improving appetitive feelings.

Chapter 6: The association between sugar sweetened beverage intake and reward learning in Hispanic children

Shearrer GE, Mumford J, Pont SJ, Poldrack RA, Davis JN

GES was responsible for data collection, data analysis, and manuscript preparation.

ABSTRACT

Background: The neurological effects of SSB, on children are unknown. Previous research shows that reward-seeking behaviors are associated with heightened prediction error responsivity. However this has not been tested in young children, nor with SSB rewards. The objective of this project was to evaluate the effects of sugar sweetened beverage consumption on the neural response to reward prediction error (PE) in overweight Hispanic children. We hypothesized low SSB drinkers would exhibit an increased sensitivity to SSB prediction error compared to high consumers.

Methods: Overweight or obese, Hispanic youth (7-10 y) performed a probabilistic reward paradigm in a functional magnetic resonance imaging (fMRI) scan. There were 24 blocks of task where each block consisted of showing the subject a picture of a SSB or water, followed by the oral delivery of either SSB or a tasteless solution. In 80% of the blocks the cues matched the beverage and in 20% of the blocks they did not. This strategy allowed for estimation of neural response to PE, which is considered a correlate of dopaminergic signaling.

Results: A total of 32 scans were included in this analysis. The average age of the subjects was 8.8 y and the mean SSB intake was 0.5 servings per day. Due to high motion, the data was not interpretable.

Discussion: this study provided a wealth of methodological data. Children 7 to 8 may be too young to preform taste based functional tasks. Additionally, mock scanning may be a useful solution to minimize motion in older children (9-10 y) in future studies.

INTRODUCTION

Hispanic children in the United States are at a disproportionate risk for obesity and type 2 diabetes [363]. A potential cause of the disparity between Hispanic children compared to NHW children could be SSB consumption. SSBs are the primary source of added sugar for US children (2-18 y) [57]. For every serving of SSB per week odds of becoming obese increased 29% in Hispanic children (8-10 y) [117]. A study with 770 Korean children showed a positive relationship between consumption of sugar from SSB and incidence or prevalence of metabolic syndrome [364]. The increase in risk was seen only between increased sugar from SSB, not from total sugar intake, or from sugar consumption from fruit [364]. While overall, the national consumption of carbonated SSB has decreased, in 2014, consumption of SSBs still makes up over half of all beverage consumption in children (2-19 y) [99]. The World Health Organization recommends limiting added sugar consumption to less than 10% of total caloric intake [365]. A single serving of SSB, approximately 50 grams, of sugar easily exceeds this recommendation [72]. The prominent sweetener in SSB is high fructose corn syrup (HFCS), which on average, contains as much as 60% fructose [72]. Compared to glucose, intake of pure fructose has been implicated in increased cardio-metabolic risk factors as well as in an increase in liver fat [74,75,135,137,210,366].

Cognitive systems have been implicated in nutrient intake, feelings of satiety, and weight status [21]. Desire to eat pleasurable foods has been noted as a barrier to weight loss in children [22,23]. Hedonic reward has previously been posited to override homeostatic hunger and satiety inputs leading to over consumption of palatable foods and drinks despite physiological signaling to stop eating, ultimately leading to weight gain [16]. Reward is a multifaceted psychological component comprising learning, incentive motivation, and pleasure [24]. Learning about reward is encoded in the brain through a reward prediction error signal further programmed through dopamine signaling [25–28]; when a stimulus is better than expected it results in higher dopamine response, when a stimulus to poorer than expected it attenuates the dopamine response [29]. Therefore, the effect of the stimulus when it is different than expected is a useful measure of the signals that underlie reward related learning [30]. In an animal model, intermediate sucrose-sweetened drink intake was found to impair food-related learning, and this impairment of learning was recovered with administration of a dopamine 2 receptor agonist [63]. This suggests that daily intake of a sucrose beverage (similar to commercially available soft drinks) alters the ability to learn about other food rewards, and appears to mediated via dopaminergic signaling.

Increased body mass index can be considered a symptom of prolonged palatable food intake, therefore one would expect to see similar differences in reward based learning and dopaminergic activity [367]. FMRI allows for the visualization of the BOLD response, a correlate of neuronal activity [30]. Previous studies have shown that overweight children (8-12 y) display higher activity in the amygdala and insula in response to a sweet

taste [368], and obese adolescents have shown increased striatal and limbic response to high calorie foods compared to lean adolescents [369]. After eating, obese compared to lean children (10-17 y), did not exhibit a diminished signal in response to a food cue [370]. Chronic intake of highly palatable food also appears to modulate the brain's response to food cues. Frequent ice cream has been related to a reduction in reward sensitivity in healthy weight teenagers [371], and this reward responsivity was further related to weight gain in teenagers [256] and eating in the absence of hunger [250]. These studies suggest that chronic intake of high calorie foods alter the brain's response to food cues in human youth. Brain response to high calorie and low calorie foods in children (7-10 y) was found to be positively associated with weight status [372]. Only one previous study from Burger and Stice has looked at the effect of a highly palatable HFCS drink on reward responsivity [373] and the present study builds on their work, but uses a younger population and specifically examines the effect of regular HFCS intake on prediction error signals.

Examining prediction error response to a HFCS stimulus to assess reward sensitivity and possible risk for weight gain and eating in the absence of hunger is particularly important in children (7-10 y). The prevalence of overweight or obesity in Hispanic children jumps from 29.8% in children (2-5 y), to 46.2% in children (6-11 y) [5], indicating a period of high risk for the development of high BMI, which has been linked to significant health complications as adults [374]. In addition, this is an age range that is moving toward more autonomous eating and food/beverage choice by the child. Understanding what and how behavioral factors are linked to this dramatic increase in obesity in this developmental age is warranted.

Therefore, the goal of this pilot study was to examine and compare the effect of a HFCS receipt prediction error signaling in brain response using fMRI in Hispanic children (7-10 y). We hypothesized participants who consume more free-living SSBs will show a depressed positive prediction error response and an increased negative prediction error response.

SUBJECTS AND METHODS

Participants

Participants were recruited from local health clinics and by word of mouth around the Austin area. All participants had to be Hispanic (7-10 y) (four grandparents of Hispanic descent), and be overweight or obese (85^{th} percentile ≤ BMI). Participants were excluded from the study if they had MR contraindications, were taking medications known to influence body composition, had type 1 or 2 diabetes, and or had any major illness known to influence body composition. The participants were also screened for disordered eating with the "Questions about making your self Sick, loss of Control, loss of One stone in three months, do you believe you are Fat, and would you say Food dominates your life" (SCOFF) eating disorder screening tool adapted for children in the United States (23). The Institutional Review Board of the University of Texas at Austin approved this study. Written informed consent was obtained for all participants from their legal guardians, as well as written assent from the participant before any testing was done.

Body Composition

Height and weight were measured to the nearest cm and kg on a seca-769 physician scale and stadiometer (Seca, Chico, CA).

Dietary Recalls

Three multi-pass 24-hour recalls using NDS-R software (2014; Nutrition Data System for Research, University of Minnesota, Minneapolis, MN) was collected via telephone to assess free-living dietary intake. All three dietary recalls were performed within one week of the in-person testing visit. SSB categories included the following variables: carbonated sweetened drinks, energy drinks, sports drinks, sugar flavored milk, sweetened coffee drinks, sweetened teas, and juice drink.

Testing Visit

All testing visits started between 7 and 9 am. Participants were instructed to come to the testing visit after a 12 hour fast, nothing to eat or drink except water. Participants were called and texted the evening before the visit to remind them when to start fasting.

Stimuli and Delivery

The stimulus was a common fruit flavored drink, Tampico, which contains 15 kcal/fl oz. According to recent high-performance liquid chromatography study, Tampico contains 11.4 grams of sugar per 100 ml and is sweetened with HFCS that is 58% fructose [73]. Tampico was selected as the taste stimulant as it is culturally appropriate to Hispanic children in Central Texas. A tasteless solution composed of 2.5 mM sodium bicarbonate and 25mM potassium chloride at a 25% dilution, designed to mimic saliva, was used as a control taste. The Tampico and tasteless solution were delivered during

scanning via a gustatory manifold positioned on a plastic reticulated arm connected to the scanner bed (½" diameter Loc-Line, Lockwood Products, Lake Oswego, OR). The manifold was connected to 2 BS-8000 syringe pump system (Braintree Scientific, Braintree, MA). Each pump was fitted with a 60ml syringe, one with Tampico and the other with tasteless solution. The pumps delivered liquid to the manifold via Tygon beverage tubing (Saint-Grobain Performance Plastics, Akron, OH). This allowed the manifold to drip 0.5ml of either Tampico or tasteless solution onto the participant's tongue.

Functional Task

The beverage task used a probabilistic cueing paradigm. A single trial of the task is illustrated in **Figure 6.1.** On each trial, participants were first presented with a 2 second cue indicating the beverage that will be delivered (a picture showing either a bottle of Tampico or a bottle of water). After the 2s long cue, 0.5 ml of either beverage was delivered via the gustatory manifold over 2 seconds, followed by 2s waiting period, then a 2 second rinse with 0.5 ml of the tasteless solution and, finally, a 2s long swallow cue. An 2s intertrial interval separated each trial. There were 24 trials in each of 2 runs for each participant. The cue and delivery were 80% valid, such that on 20% of trials, subjects received a cue that differed from the beverage that was actually delivered. This paradigm was chosen to best measure prediction error signals at receipt.

Functional MRI Data Acquisition

MRI data was collected using a Siemens Skyra 3T scanner with a 32-channel head coil at the UT Imaging Research Center. FMRI acquisition involved 36 contiguous axial slices with isotropic 3mm voxels, collected at an oblique angle to improve signal from ventromedial prefrontal regions and amygdala, using an echo-planar acquisition (repetition time (TR) = 1.59; echo time (TE) = 30 ms; flip angle = 90; 64 x 64 matrix; field of view=192 mm, acceleration factor=2). After the beverage task, high-resolution T1-weighted volumetric scans using a magnetization-prepared rapid-acquisition gradient echo sequence (MP-RAGE) for anatomical registration were collected.

Image Preprocessing

FMRI data was analyzed via FSL 4.2.0 (FMRIB's Software Library). Brain extraction tool (BET) and MCFLIRT were be used for skull extraction and motion correction, respectively. Scans with TRs showing an absolute mean displacement greater than 3mm were excluded from analysis. Confound motion parameter regressors were created for TRs with a framewise displacement (FD) greater than 0.9, such that each high motion TR was modeled using an indicator for the high motion TR (1 for the TR of interest, rest of TRs modeled with 0), effectively removing signal specific to those timepoints. These FD-based motion regressors were modeled along with the 24 regressor extended motion parameter set that included the 6 motion parameters, their squares and the derivatives of each. A functional run for a subject was excluded if more than 20% of all the volumes were tagged as high motion. Highpass temporal filtering, using a

Gaussian weighted running line smoother, removed low frequency drift with a 100s cutoff. Individual data were spatially smoothed with a 5mm Gaussian filter. Prewhitening was achieved with FSL's FILM tool. Boundary based image registration was used to align the EPI data with the subject's structural T1 image [375] and FSL's FNIRT tool was used for the registration of the subject's structural with the MNI152 template, using a warp resolution of 10mm. Runs within subject were combined using a fixed effects model and group level analyses used the FMRIB Local Analysis of Mixed Effects (FLAME 1) model in FSL [376].

Functional Data Model

The model was composed of eight regressors. Two regressors modeled the cue, one modeled when the water bottle image was seen and one modeled when the Tampico bottle was seen during the two-second cue period. Four regressors modeled the potential cue and taste combinations. Of the four cue taste combinations, two modeled the matched cues and tastes during the 2-second taste delivery period: water cue, neutral taste; Tampico cue, Tampico taste. Negative prediction error was modeled during the 2-second taste delivery period: Tampico cue, neutral taste. Positive prediction error was modeled during the 2-second taste delivery period: water cue; Tampico taste. Additionally, the rinse delivery was modeled during the 2-second rinse delivery, and the swallow cue was modeled during the 2-second swallow cue period (**Figure 6.1**).

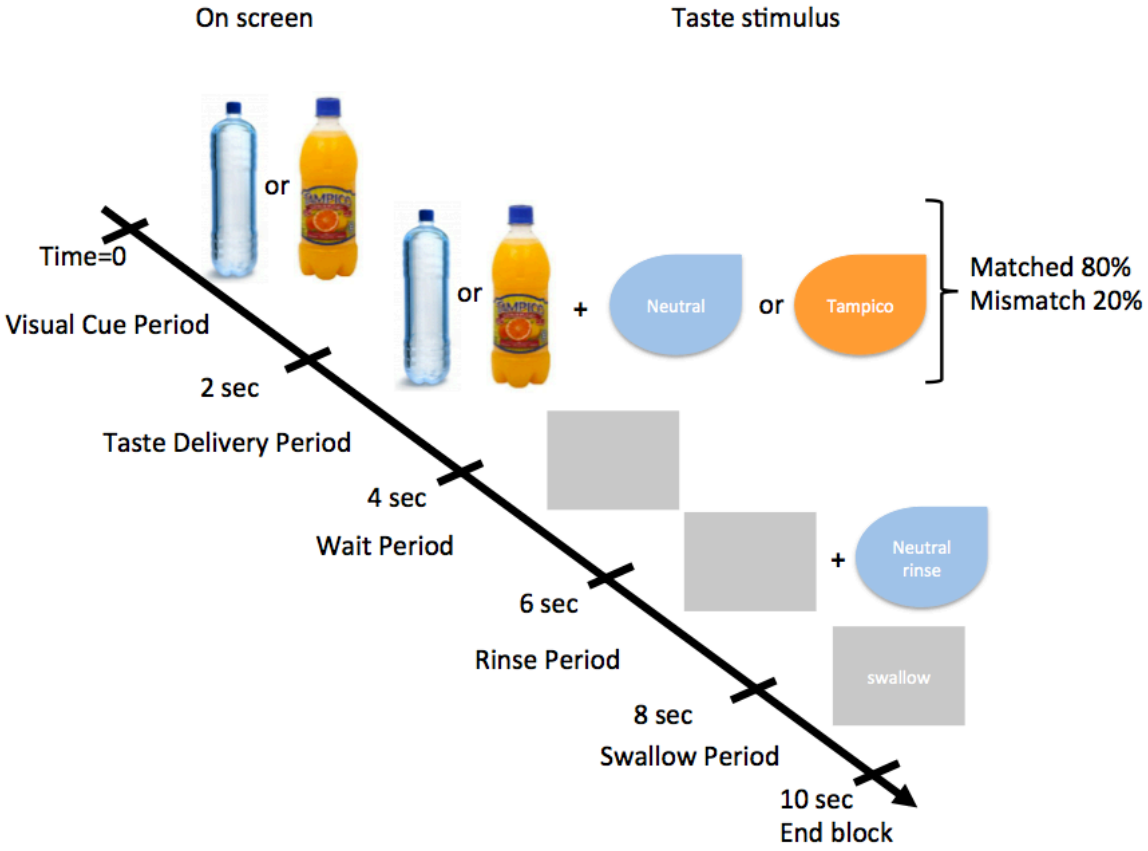

Figure 6.1: Diagram of the reward prediction error paradigm used while scanning. Children were shown a picture of either Tampico or water, followed by a 0.5 mL taste of Tampico or a neutral solution. There was then a wait, a neutral solution rinse, and a swallow cue.

The difference between cues was modeled as the water cue minus the juice cue (cue_{water}-cue_{juice}). The difference between positive prediction error and negative prediction error was modeled as positive prediction error minus the negative prediction error ($PE_{positive}$-$PE_{negative}$). The difference between prediction error and matched cues was modeled as positive prediction error and negative prediction error minus both matched water cue and taste and the Tampico cue and taste ([$PE_{positive}$+$PE_{negative}$]-

[$Matched_{water}$+$Matched_{Tampico}$]). The difference between matched cue and taste were modeled as Tampico cue and taste minus water cue and taste ($Matched_{tampico}$+$Matched_{water}$). Finally positive and negative prediction error were modeled against baseline (the 2-second wait period). All regressors were convolved with a double gamma hemodynamic response function to model the delayed BOLD response.

In the group level model, SSB intake (measured as 8oz servings per day) was log10 transformed and added to the group level model as a continuous covariate. Nonparametric permutation testing was preformed using FSL's randomize using the threshold free cluster enhancement (TFCE) algorithm with 5000 permutations [377], with a corrected threshold of $p<0.05$.

Additionally small volume corrections (SVC) were used with the following a priori regions of interest (ROIs) to examine predicted effects: orbital frontal cortex [378–380]nucleus accumbens [378,380–382], amygdala [380,383], insula [77,384], caudate [77,254,385], and putamen [380], these were defined using the Harvard-Oxford subcortical and cortical probability atlases (FMRIB, Oxford, UK). For all SVC analyses significance was set at $p<0.05$. Nonparametric permutation testing using FSL's randomize using the threshold free cluster enhancement (TFCE) algorithm with 5000 permutations [377] was also preformed for all ROIs. Coordinates are reported in MNI with cluster size (k) in voxels.

RESULTS

A total of 32 subjects were included in this analysis. All subjects were overweight or obese (mean BMI percentile 95.8 ±3.8) with 17 female and 15 male. Twenty-two of

the subjects were obese (BMI ≥95th percentile for age and sex). The flow of participants through the experiment can be found in **Figure 6.2**.

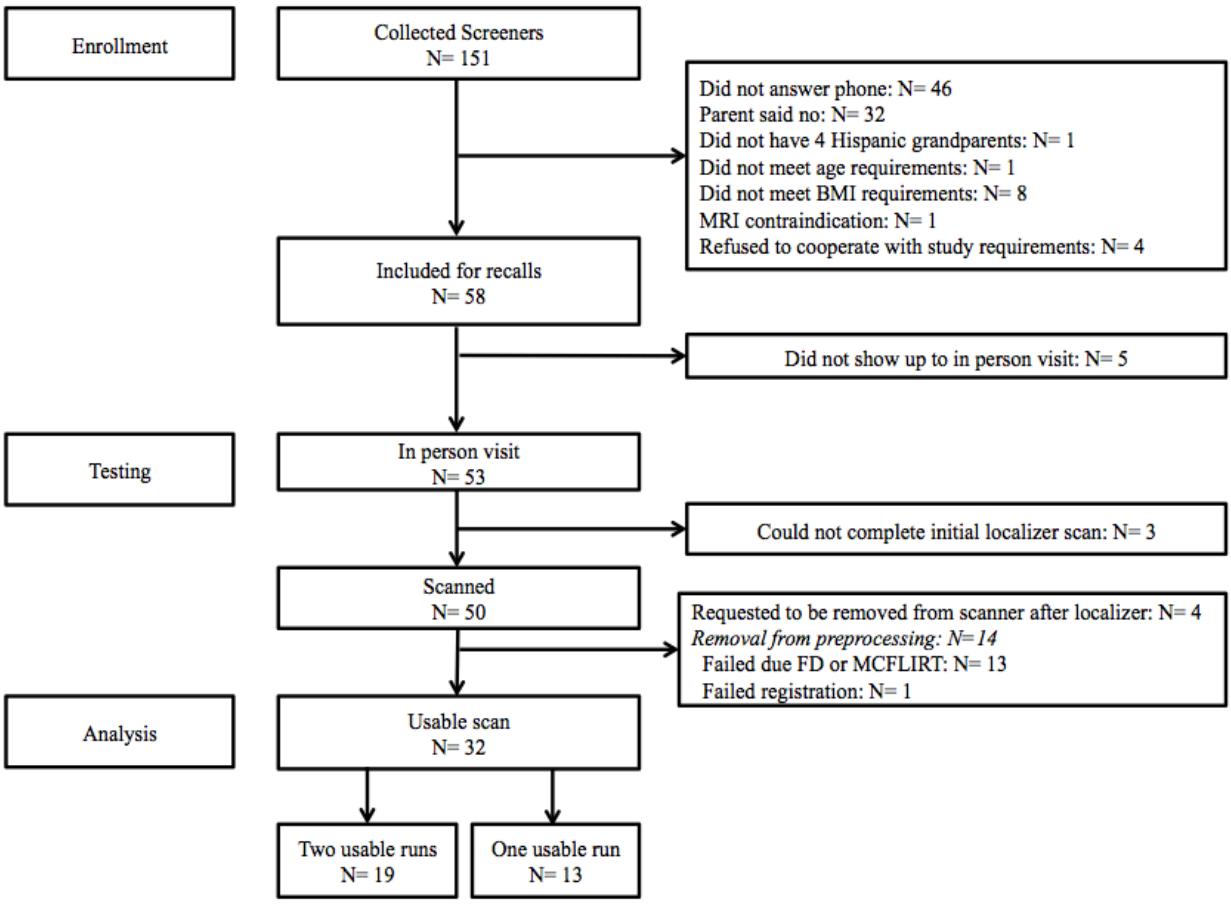

Figure 6.2: Consort diagram showing the flow of participants through various stages of data collection. Framewise displacement (FD); Motion correction FMRIB's linear image registration tool (MCFLIRT).

A total of 53 subjects attended the in person visit; of those three were unable to complete the initial localizer scan. Fifty subjects completed the functional task. Of the 50 subjects who completed the functional task, 32 subjects had data that passed preprocessing quality checks. Nineteen subjects had two usable runs and 13 had only one

usable run. One subject's anatomical scan exhibited substantial artifacts and therefore was not able to be registered to an MNI-152 template and was excluded. The average age was just under 9 years (8.8 ±1.1 y). On average the subjects consumed 1501.3 calories daily and less than one serving of SSB per day (0.7 ±0.5 servings per day).

The pattern of BOLD response in the positive and negative prediction error models follows the ventricles and edges of the brain and is therefore suggestive of a motion artifact and uninterruptable (**Figure 6.3**). Unfortunately, since the prediction error tasks compare BOLD response to baseline, the other contrasts are also likely skewed due to motion artifacts.

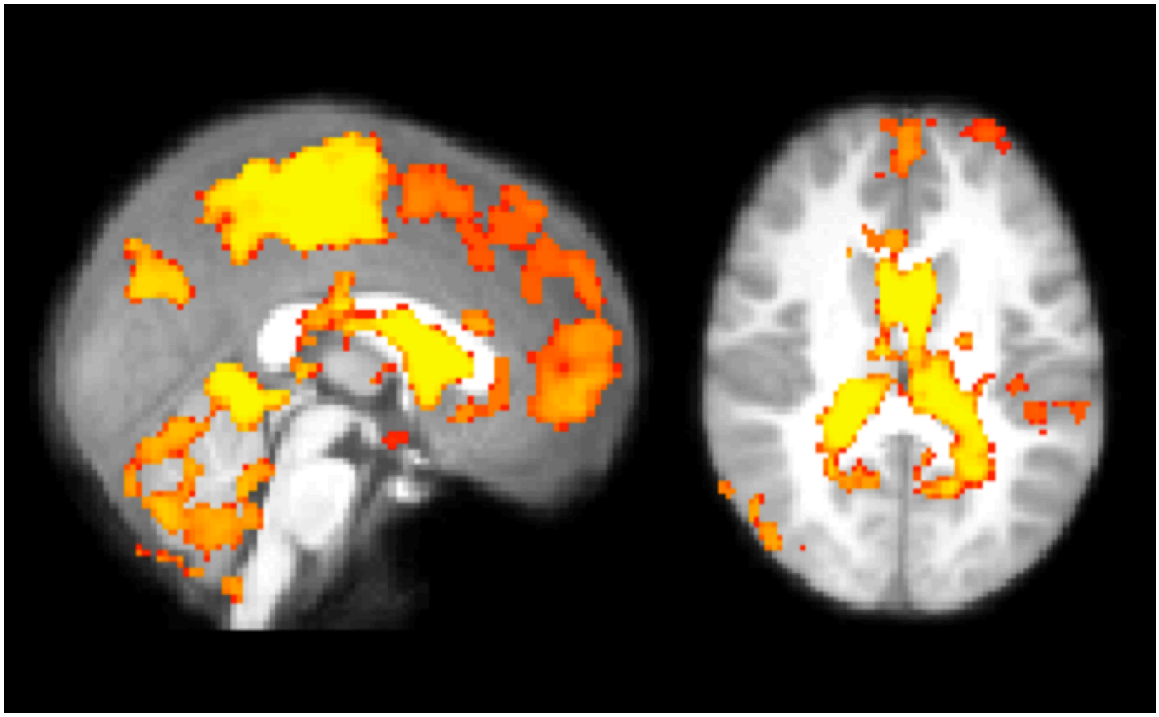

Figure 6.3. Statistic maps generated from Randomise based on the threshold-free cluster enhancement (TCFE). Activation in the ventricles and near the edges of the brain indicates noise from motion.

DISCUSSION

Although this pilot study did not yield viable data on our hypothesis, it does provide valuable methodology information that will inform future studies in this population. This was the first study to use a taste task in young children (7-10 y). Boredom during the task, unease of the magnet environment, discomfort during the functional task, and fidgeting may have all been potential sources of movement resulting in the motion artifacts. Future studies should look into smaller, child sized gustatory manifold as some of the discomfort may have arisen from the gustatory manifold being too large. The children may also benefit from practice sessions drinking from the gustatory manifold before the task. In the current study this was not feasible due to time constraints. The families of the participants were predominately lower income and therefore were not able to come to multiple visits to practice using the mock scanner due to work scheduling conflicts and inability to get transportation to the UT campus. Additionally, because this study required the child to be fasted, training before the actual scan was not possible as the child would have become too hungry. Finally, time available to use the scanner was a constraint. A high quantity of weekend scanning, often with back to back scans did not allow for the child to redo scans where motion was suboptimal. Additionally, subjects were often late arriving to scanning sessions further limiting scanner availability time. A common problem was the child believed he or she was being still when in reality he or she was still moving enough to disrupt the data. Haptic feedback may be a good way to minimize motion and can be achieved by placing a small strip of double stick on the head coil, which also sticks to the child's forehead.

When the child moves his or her head they feel the slight pull from the tackiness from the double stick tape, when still he or she should not feel any pull. This way the child can feel when their heads move within the head coil and can be cognizant of movement. Again, training sessions using a mock scanner would be a useful way to implement the hepatic feedback training. Mock scanning in this age range has been shown to be a feasible way to minimize motion [372,386–389]. A drawback to this however, is small children's heads do not fill the head coil so the tape would not touch both their heads and the coil. This issue may be ameliorated with more padding or a pediatric head coil. Finally, a major limitation of this pilot study was a small sample size.

Future studies should aim for larger samples. Of the 50 subjects who completed scans, 32 passed initial quality assurance checks. Therefore, to get 50 subjects who passed the quality assurance check a more reasonable sample would contain at least 80 subjects. Furthermore, the population of this study may have been too narrow. Given that 151 screeners were collected for 32 scans, at least 380 screeners need to be collected to theoretically get 80 scans. Expanding the recruitment criteria to other races and ethnicities or including lean children may be a strategy to increase sample size and scan quality.

The mean age of the subjects who were able to complete 2 scans was 9.1 ±1.1 y, this suggests that older subjects are able to better complete the functional task. Future studies should test the functional task in a slightly older population to see if age may be a precluding factor. Ideally, this task should be testing in a group of subjects ages 9 to 15 y as similar tasks have been shown to work in adolescents [234,238,369,390]. Testing both

adolescents and children may provide insight into what strategies older children use in the magnet and where the age limit of feasibility occurs. An interesting possibility with scans with adolescents and children would be allowing the younger children to preview a scan to alleviate unease in the scanner environment.

To conclude, while the scanning data collected was not interpretable, this study provided critical insight into pediatric functional task scanning. Children under the age of 8 y do not appear to be able to perform functional tasks involving drinking. Additionally, in order to get larger sample sizes, oversampling may be required. Mock scanning and specialized pediatric gustatory manifolds may improve scan quality by reducing motion during the taste administration. However these methods require multiple testing visits, which will require increased incentives and may not be possible in low-income populations. Research with both children and adolescents would be valuable to assess the differences between a population who has shown the ability to complete a taste task and a younger population.

Chapter 7: Conclusion

This research focused on elucidating the relationships between sugar sweetened beverage (SSB) consumption and metabolic health in high-risk and understudied populations: Non-Hispanic Black (NHB) and Hispanic youth[2,4]. Specifically, this dissertation examined the interplay between perceived hunger and satiety, endocrine balance, fat partitioning and SSB consumption. Analysis of cross-sectional data from two studies conducted at University of Southern California (USC) revealed high free-living SSB intake appears to be related to a decrease in perceived fullness at an ad libitum test meal and lower ghrelin response in overweight and obese NHB and Hispanic adolescents (14-17 y). Furthermore, SSB consumption was found to be independently associated with higher visceral adiposity (VAT) and higher cortisol awakening response (CAR) in a similar sample of overweight and obese NHB and Hispanic adolescents (14-17 y). In a cross-sectional study conducted at the University of Texas (UT) with a younger sample of overweight and obese Hispanic children (7-10 y), added sugar intake was associated with increased hunger and decreased satiety at an ad libitum test meal. Interestingly, in the younger child population no homeostatic factors were related to hunger or satiety. This suggests, that hunger and satiety in children is not homeostatically motivated, but rather is hedonically driven. Although we had planned on further exploring the hedonic aspect of hunger and reward learning in this population using functional magnetic resonance imaging (fMRI), we were unable to interpret the data gathered due to motion artifacts. Regardless, the results from the above studies indicate that in adolescents SSB intake is related to a decrease in satiety, altered metabolic

hormones, and increased VAT accumulation. Younger children do not appear to show the same relationship between satiety and metabolic hormones; rather availability of highly palatable foods is closely related to perceived satiety. Reduction in added sugar availability in young children and limiting SSB intake in adolescents may help increase satiety and in adolescents improve metabolic health.

The work presented here supports the hallmarks of Carnell and Wardle's obese phenotype hypothesis: low responsivity to internal satiety signaling (homeostatic hunger) and increased responsivity to external food cues (perceived hunger) [140] with the added caveat that dietary intake can influence both internal satiety signaling and external food cues. Previous work has suggested that SSB increases caloric intake, greater than that achieved through SSB intake alone, and therefore SSB may suppress satiety or increase appetite [144]. However, previous research also has focused on acute intake of a dietary variable on appetite [139,287,288,290,291]; but it appears that free-living SSB intake decreases satiety signaling in overweight and obese adolescents.

While ghrelin is known as a "hunger hormone" and lower ghrelin would suggest lower hunger, the subjects who consumed high free-living SSB also exhibited reduced satiety compared to their low free-living SSB consuming peers. The reduction in circulating ghrelin may indicate a potential mechanism for the reduced feelings of satiety related to SSB intake. The high SSB consumers compared to the low may be exhibiting hunger uncoupled from homeostatic mechanisms. Therefore, even with low hunger signaling (ghrelin), the high SSB consumers still feel less satiated. The blunted ghrelin response may be an indicator of dysregulated satiety peptides. Depressed circulating

ghrelin has been found in obese humans compared to lean [32], however the results of Chapter 3 show that within overweight and obese humans SSB intake further suppresses ghrelin. Aberrant circulating ghrelin may be symptomatic of a host of other abnormal metabolic responses[391], such as hyperinsulinemia [166,180,183–185]. Insulin has been shown to reduce ghrelin secretion in adults [180,184] and children [166,183,185]. Under normal conditions insulin is a satiety hormone [392]. The HELENA study showed that SSB consumption was positively related to HOMA-IR in European adolescents [133]. Together, the insulin resistance seen in the HELENA and the suppressed ghrelin signaling in Chapter 3 implies SSB consumption results in metabolic and hedonic habituation to these hormones.

Along with decreased sensations of fullness and suppressed ghrelin, SSB consumption was associated with an increase in VAT and CAR independent of sex, age, caloric intake, BMI, and perceived stress in Chapter 4. Previously, cortisol has been implicated in fat partitioning[43,225]. Animal models have shown that glucocorticoids impair normal adipogenesis favoring VAT production [225]. Additionally, glucocorticoid receptors in VAT have been linked to heightened local cortisol production and receptor expression [43], creating what appears to be a positive feedback loop. However, the effect of diet on both CAR and VAT is not well known. Gyllenhammer and colleagues found that total and added sugar intake increased the relationship between cortisol and VAT in a similar sample of NHB and Hispanic adolescents [228]. A possible mechanism for both the results in Chapter 4 and in Gyllenhammer's study could be cortisol influencing satiety and dietary intake. Treatment with exogenous glucocorticoids has been shown to increase food intake [52], and chronic glucocorticoid exposure has been related to increased

palatable food intake[322,323], in particular from sweet foods compared to savory foods [324]. The increased desires to consume sweet foods, such as SSB, could be providing additional calories responsible for the increase in VAT.

Considering the depressed ghrelin secretion in high SSB consumers seen in Chapter 3, and the association between suppressed ghrelin and hyperinsulinermia, another possible mechanism linking VAT, CAR, and SSB intake could be insulin sensitivity. In Hispanic children (8-13 y), cortisol was shown to be negatively associated with insulin sensitivity[224] and metabolic syndrome was associated with increased morning cortisol [46]. Additionally, high free urinary cortisol has been associated with insulin resistance, LDL, HDL, and VAT in overweight girls (12-18 y) [45]. More analysis is needed to examine insulin as a possible mediating factor between CAR, VAT and SSB.

A critical aspect between the relationship between SSB intake, CAR, VAT, and metabolic health could be the sweetener used in SSB. Adolescents are the highest consumers of fructose, and this is thought to be in large part due to their high SSB consumption [136]. A large study of 559 adolescents ages (14-18 y) found that fructose intake was related to VAT, and that VAT mediated fructose related associations with cardiometabolic risk factors [137]. In the current study we did not examine specific types of sugar in the SSB, but the SSBs are have found to contain up to 65% fructose [72,73]. Stanhope and colleagues have shown fructose beverages, compared to glucose beverages balanced for caloric load, to be positively related to increased VAT[75]. Why fructose is detrimental to metabolic health in large part has to do with how it is metabolized at the level of the liver. Initially, fructose is taken up from the portal vein into the liver, where

unlike glucose, it by-passes the rate limiting step of phosphofructokinase. Then fructose is rapidly metabolized by ketohexokinase, which links to glycolysis through adolase. The lack of a rate limiting step overloads the glycolytic path way and increases de novo lipogenesis while suppressing hepatic insulin sensitivity [75,210]. Additionally, fructose consumption up regulates very low lipoprotein (VLDL) production in the liver to transport triglycerides synthesized from de novo lipogenesis; these VLDLs preferentially store triglycerides in VAT [75,210], which in turn is related to a decrease in ghrelin [32,180]. Cortisol may aggravate this process, especially in conjunction with VAT. Fructose consumption has been shown to increase macrophage infiltration of VAT, which may be responsible for the increasing pro-inflammatory signaling and decreasing anti-inflammatory signaling from adiponectin [210]. Cortisol additionally promotes adipogenesis [225], induces hyperleptinemia further antagonizing adiponectin [226], as well as increases triglyceride uptake in adipocytes by up-regulating lipoprotein lipase [43]. Therefore the high fructose intake decreases hepatic insulin sensitivity, increases VLDL, and increases VAT inflammation (through increased macrophage infiltration and decreased adiponectin). This state of inflammation may signal increased cortisol production, which in the obese state leads to increased triglyceride uptake from VLDLs, preferential creation of VAT, and increased circulating leptin, further antagonizing adiponectin and insulin. The combination of high CAR, VAT, and high fructose intake from SSB may create a metabolic storm, particularly in the liver. Overall, SSB intake is related to unfavorable metabolic phenotypes, which may interact. Chapters 3 and 4 suggest a single bottle of SSB, available from any vending machine, is associated with increased VAT, CAR and

decreased ghrelin and fullness in overweight and obese NHB and Hispanic adolescents (14-17 y).

Unlike in adolescents, in overweight and obese Hispanic children (7-10 y), free-living SSB intake was not related to appetite measures, nor was it related to cortisol response, insulin response, or adipokines. However, added sugar intake at an ad libitum meal was associated with hunger and satiety at the ad libitum meal, independent of BMI, caloric intake, or endocrine signaling. A possible reason for the dissimilar results is age or pubertal status. It could be that metabolic changes associated with SSB intake do not manifest until adolescence or post pubertal stages. Zheng and colleagues found that adolescents (15 y), but not younger children (9 y), had large increases in BMI associated with SSB consumption [116]. The subjects in Chapter 5 also consumed far less SSB (mean intake 0.5 servings per day) compared to the adolescents in Chapters 3 and 4. Lower SSB intake among younger children is not a surprise. High school students have been shown to consume higher amounts of SSB compared to middle and elementary school children [100]. Puberty is also a critical element to consider when comparing young child populations and adolescents. Insulin sensitivity declines during puberty in overweight Hispanic youth [9,393,394]. This suggests that intervention in younger children, when insulin sensitivity is more favorable, may be critical to prevent the metabolic disturbances seen in Chapters 3 and 4.

Chapter 5 further supports Carnell and Wardle's obese phenotype hypothesis showing that that obese children are more responsive to external cues to eat. This is particularly relevant as previous research in children (5-6 y) showed poor appetite regulation was

associated to increased susceptibility to obesegenic environments [329]. Additionally, Fisher and colleagues found that in Hispanic children (5-18 y), appetitive (hunger and satiety) behaviors were highly heritable, and that obese children were more likely to eat in the absence of hunger [331]. Combined with the current findings, this suggests that Hispanic children may be facing a two-pronged satiety dysregulation: feeling hungrier to added sugar food cues, as well as consuming energy when not hungry.

A possible mechanism for the increase in hunger and decrease in satiety with increased added sugar intake is how people view the satiating aspects of certain foods. In a study with adults, high sugar and high fat foods were rated as being more satiating compared to low sugar and low fat snack with similar energy content [353]. Children who felt hungrier at the ad libitum meal may have considered the high sugar snacks as more satiating and therefore preferentially consumed more added sugar snacks compared to those who felt less hungry. In children (11-12 y), the satiating qualities of foods appear to be related to the child's familiarity with the food [355]. Therefore the children who felt hungrier may also have been familiar with foods with high added sugar content. This could in part be related to the increased advertisement of high sugar foods targeted at children [356]. Additionally, previous research has shown that children with greater appetites and decreased ability to control satiation consume bigger portions [329], which has in turn been linked to increases in BMI [350]. Additionally, many studies in children have compared overweight and obese children to lean children, showing obese children (5-7 y [351] and 7-12 y [352]) exhibit lower internal satiety cues compared to lean children. Synthesizing this research together, children who are overweight and obese have poorer

appetite control compared to lean children and when given options to satiate hunger, children will choose foods they are familiar with, which commonly are higher in added sugar.

The children at the ad libitum meal also consumed nearly their entire day's worth of added sugar at a single meal. The subjects at the ad libitum meal consumed 87% of their free-living added sugar intake. The high intake of sugar in a single sitting is most likely due to two factors: availability of added sugar and lack of parental oversight. The children are probably not given free access to ad libitum food/beverages throughout their normal days. Therefore, when presented with ad libitum intake, they ate/drank as much as possible. This also poses a possible direction between the association of added sugar intake and satiety. Children may not be able to distinguish the difference between desire to consume a palatable food and physical hunger. Therefore, those with the highest desire to consume added sugar reported the highest feelings of hunger. An additional possible mechanism could be the lack of parental oversight during the ad libitum meal. The children may rely on parental influence for portion control, as parenting style has been shown to influence child eating behavior [358–361]. More research is needed to elucidate the influence of parenting on added sugar intake in the absence of the parent. However, overall the subjects in this study consumed high amounts of added sugar compared to daily free-living added sugar consumption and this could be driven by a desire to eat sugary foods interpreted as hunger.

In addition to the dietary, satiety, and endocrine data, fMRI data was also collected. However, due to high motion artifacts, the models described in Chapter 6 were

uninterruptable. Although conclusions could not be drawn about the effect of HFCS on prediction error, the study did provided valuable information about using a taste based fMRI task in children. This study was the first to use a taste fMRI task in children (7-10 y). Although similar tasks have been used successfully in adolescents [234,256,373,395], children appear to need a scanning environment more tailored to their age range. The motion was predominately during the taste administration. Future studies with children should use a smaller gustatory manifold, as some motion appears to be related to discomfort of the mouthpiece. Additionally, while not used here due to feasibility issues, mock scanning sessions with taste administration may improve data quality [372,386–389]. Another common comment from the children scanned was boredom during the scan. Breaks between runs may be needed in future scanning procedures, although they do lengthen scanning times and may result in difficulties in scan registration during data processing. Haptic feedback is also an attractive method to reduce motion. Children reported staying motionless, even when researchers could see them moving, indicating that children have trouble realizing when they are moving. A small piece of double stick tape on the head coil, which also touches the child's head, may be useful to indicate to the child when they are moving. When the child moves his or her head they feel the slight pull from the tackiness from the double stick tape, when still he or she should not feel any pull. This way the child can feel when their heads move within the head coil and can be cognizant of movement. However, this may be difficult in children who have small heads, which may not get close enough to the head coil. In that case additional padding may have to be used, or ideally a pediatric head coil. Further research, employing the previously mentioned

methods, should be conducted to explore the effect of HFCS on reward learning in children. While the fMRI data from this study was uninterruptable, this analysis provided critical methodological insight for planned future grant submissions.

Along with the taste paradigm used in Chapter 6, a willingness to pay paradigm was also collected. This examined the child's willingness to pay for various foods. The child was shown an image of a food and asked to push a button indicating if they would pay $0, $1, $2, or $3 for the item. The foods shown were one-third high sugar and low fat, one-third high sugar and high fat, and one-third low sugar and low fat. Many of the images shown were also offered at the ad libitum meal. This allowed the child to consider the food shown, rate the food, and then gave them the opportunity to consume the food. This data will be analyzed for correlations between intake at the ad libitum meal and metabolic biomarkers. This data may further inform what children value and how that value translates into consumption.

The cross-sectional studies presented here provide foundation for further research. Foremost, the relationship between SSB, VAT, CAR, perceived satiety, and insulin needs to be assessed in a large sample of NHB and Hispanic adolescents. Understanding the relationship between SSB, VAT, CAR, perceived satiety, and insulin would help inform future obesity prevention and treatment programs. Additional research is needed to examine the effects of long-term high fructose corn syrup exposure in minority populations. In younger children, the effect of hunger on dietary choice warrants further investigation, as well as how familiarity and advertisements influence children's beliefs

about satiety. Specifically, research is needed to explore what foods children believe to be satiating and what influences children in dietary choices to reduce feelings of hunger.

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Vita

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